



BearWorks

---

## MSU Graduate Theses

---

Summer 2017

# Effects of Engineered Carbon and Silver Nanoparticles on *Plutella Xylostella* Consumption, Growth, Pupation, Survival, and Fecundity

Taiaba Afrin

Missouri State University, [Taiaba2015@live.missouristate.edu](mailto:Taiaba2015@live.missouristate.edu)

As with any intellectual project, the content and views expressed in this thesis may be considered objectionable by some readers. However, this student-scholar's work has been judged to have academic value by the student's thesis committee members trained in the discipline. The content and views expressed in this thesis are those of the student-scholar and are not endorsed by Missouri State University, its Graduate College, or its employees.

---

Follow this and additional works at: <https://bearworks.missouristate.edu/theses>

 Part of the [Biology Commons](#)

## Recommended Citation

Afrin, Taiaba, "Effects of Engineered Carbon and Silver Nanoparticles on *Plutella Xylostella* Consumption, Growth, Pupation, Survival, and Fecundity" (2017). *MSU Graduate Theses*. 3130.  
<https://bearworks.missouristate.edu/theses/3130>

This article or document was made available through BearWorks, the institutional repository of Missouri State University. The work contained in it may be protected by copyright and require permission of the copyright holder for reuse or redistribution.

For more information, please contact [BearWorks@library.missouristate.edu](mailto:BearWorks@library.missouristate.edu).

**EFFECTS OF ENGINEERED CARBON AND SILVER NANOPARTICLES ON  
*PLUTELLA XYLOSTELLA* CONSUMPTION, GROWTH, PUPATION,  
SURVIVAL, AND FECUNDITY**

A Masters Thesis

Presented to

The Graduate College of

Missouri State University

In Partial Fulfillment

Of the Requirements for the Degree

Master of Science, Biology

By

Taiaba Afrin

August 2017

Copyright 2017 by Taiaba Afrin

**EFFECTS OF ENGINEERED CARBON AND SILVER NANOPARTICLES ON  
*PLUTELLA XYLOSTELLA* CONSUMPTION, GROWTH, PUPATION,  
SURVIVAL, AND FECUNDITY**

Biology

Missouri State University, August 2017

Master of Science

Taiaba Afrin

**ABSTRACT**

Engineered nanoparticles (ENPs) have recently become common in consumer products, and will therefore be entering into both aquatic and terrestrial systems. However, the effects of ENPs on animals is not well characterized. For example, to date there has been no research on the effects of single walled carbon nanotubes (SWCNTs) and silver nanoparticles (AgNPs) (common ENPs) on insects. I examined the effects of pure SWCNTs and AgNPs on consumption, growth, food conversion ratio, pupation, survival, fecundity, and expression of CYP6BG1 using fourth instar of diamondback moth (DBM) larvae with the following concentrations: 8.64, 17.28, 34.56, 69.12 and 138.24  $\mu\text{g/ml}$  SWCNTs; and 4.32 and 8.64  $\mu\text{g/ml}$  AgNPs. I measured CYP6BG1 expression with Real-time PCR. There were no measurable effects of SWCNT on DBM's consumption, pupation, and survival rate, but negative affect on growth, conversion rate and fecundity at the highest concentration (138.24  $\mu\text{g/ml}$ ). AgNPs had no effect on DBM growth, conversion, survival, and fecundity rate, but reduced consumption and pupation rate. In my experiments, CYP6BG1 was upregulated at concentrations of 138.24  $\mu\text{g/ml}$  SWCNTs and 4.32 and 8.64  $\mu\text{g/ml}$  AgNPs compared to control. My research indicates that there is no acute toxicity of SWCNTs on DBM, however further studies are warranted as fecundity and CYP6BG1 expression level appear to be affected. AgNPs are acutely toxic in terms of consumption, but studies are needed to confirm the hypothesis. My research provides insights and a model system for future studies on the effects ENP on insects.

**KEYWORDS:** *Plutella xylostella*, SWCNT, AgNP, consumption, growth.

This abstract is approved as to form and content

---

Dr. D. Alexander Wait  
Chairperson, Advisory Committee  
Missouri State University

**EFFECTS OF ENGINEERED CARBON AND SILVER NANOPARTICLES ON  
*PLUTELLA XYLOSTELLA* CONSUMPTION, GROWTH, PUPATION,  
SURVIVAL, AND FECUNDITY**

By

Taiaba Afrin

A Masters Thesis  
Submitted to the Graduate College  
Of Missouri State University  
In Partial Fulfillment of the Requirements  
For the Degree of Master of Science, Biology

August 2017

Approved:

---

Dr. D. Alexander Wait

---

Dr. Laszlo Kovacs

---

Dr. Kyoungtae Kim

---

Dr. Julie Masterson: Dean, Graduate College

## ACKNOWLEDGEMENTS

First of all, I would like to thank Missouri State University for giving me the opportunity to study abroad. I would like to express my deepest appreciation to the Graduate College of Missouri State University for all the support that was in need for the completion of my thesis.

I would also like to express my deepest gratitude to the Department of Biology. Without their help and counsel the completion of this work would have been extremely difficult.

I am grateful to my advisor Dr. Alexander Wait, whose understanding, expertise, generous guidance, and support made it possible for me to work on this topic that was of great interest of me. He is the source of insight and inspiration for this thesis.

I am highly indebted and grateful to Dr. Laszlo Kovacs and Dr. Kyoungtae Kim for readily agreeing as my advisory committee members. I would like to take this opportunity to thank Dr. Laszlo Kovacs for advising and permitting me to utilize his lab for my experiments which are very important for the thesis. I would like to thank Dr. Kyoungtae Kim for providing indispensable advice and support on different aspects of my thesis.

I thank my parents for inspiring me through their hard work. I am indebted for their support throughout while I am away from home, for giving me freedom to explore the world, and to catch my dreams.

I am thankful to my younger brother Tanjim who provides me support throughout the hardest time in my life.

I am especially grateful to my husband, Raiful for his enduring support and encouragement to continue my studies and explore my dreams.

I dedicate this thesis to my Mom and Dad.

## TABLE OF CONTENTS

Introduction.....	1
1.1. Introduction to nanotechnology and nanoparticles.....	1
1.2. Carbon Nanotubes.....	2
1.3. Silver Nanoparticles.....	4
1.4. Neutral Effects of SWCNT on Animals.....	6
1.5. Negative Effects of SWCNT on Animals.....	8
1.6. Neutral Effect of AgNPs on Animals.....	11
1.7. Negative Effects of AgNPs on Animals.....	11
1.8. Measurement of Gene Expression of CYP6BG1 .....	14
1.9. Research Goals and Questions.....	16
Materials and Methods.....	18
2.1. Single Walled Carbon Nanotube Properties.....	18
2.2. AgNPs Properties.....	18
2.3. Artificial Feed Preparation.....	19
2.4. DBM Rearing.....	20
2.5. Artificial Feed Assays.....	20
2.6. Measurement of Fecundity.....	21
2.7. Total RNA Extraction, Purification and Quantitation and cDNA Synthesis...21	
2.8. Quantitative Real Time PCR Analysis.....	22
2.9. Statistical Analysis.....	23
Results .....	25
3.1. Effect of SWCNT on DBM .....	25
3.2. Effect of AgNP on DBM.....	28
3.3. Effect of ENPs on CYP6BG1 gene expression level.....	29
Discussion .....	30
4.1. Effect of SWCNT on DBM.....	30
4.2. Effect of AgNPs on DBM.....	32
4.3. ENPs effect on CYP6BG1 gene expression level.....	33
References.....	35
Appendix.....	69

## LIST OF TABLES

Table 1. The concentrations of single walled carbon nanotube (SWCNT) and AgNP tested and the number of replicates for each feeding trial.....	54
Table 2. Primer design and properties used for qRT-PCR .....	54
Table 3. Mean ( $\pm$ SE) consumption by diamondback moths (DMB) of feed without and with increasing concentrations of SWCNTs across six trials.....	55
Table 4. Mean ( $\pm$ SE) growth of diamondback moths (DMB) feeding on artificial feed without and with increasing concentrations of SWCNTs.....	56
Table 5. Mean ( $\pm$ SE) % of pupation of diamondback moth (DBM) across six different trials for different SWCNT concentration (0 $\mu$ g/ml, 8.64 $\mu$ g/ml, 17.28 $\mu$ g/ml, 34.56 $\mu$ g/ml, 69.12 $\mu$ g/ml, and 138.24 $\mu$ g/ml).....	57
Table 6. Mean ( $\pm$ SE) % of survival of DBM across six different trials for different SWCNT concentration (0 $\mu$ g/ml, 8.64 $\mu$ g/ml, 17.28 $\mu$ g/ml, 34.56 $\mu$ g/ml, 69.12 $\mu$ g/ml, and 138.24 $\mu$ g/ml).....	58
Table 7. Percentage of $\mu$ g growth per $\mu$ g consumption for Diamondback Moth across six different trials. ....	59
Table 8. Number of eggs per adult for Diamondback Moth across three different trials.....	59
Table 9. Table 9: Mean ( $\pm$ SE) data for different variables of Diamondback Moth across six different trials.....	60
Table 10. Mean ( $\pm$ SE) consumption rate of AgNPs on DBM across two different trials.....	60
Table 11. Mean ( $\pm$ SE) growth rate of Diamondback Moth across two different trials among different AgNPs concentration (0 $\mu$ g/ml, 4.32 $\mu$ g/ml and 8.64 $\mu$ g/ml).....	61
Table 12. Mean ( $\pm$ SE) pupation (%) rate of Diamondback Moth across two different trials.....	61
Table 13. Mean ( $\pm$ SE) survival (%) rate of Diamondback Moth across two different trials.....	61



Table 14. Percentage of $\mu\text{g}$ growth per $\mu\text{g}$ consumption for Diamondback Moth across two different trials among different AgNPs concentration (0 $\mu\text{g/ml}$ , 4.32 $\mu\text{g/ml}$ and 8.64 $\mu\text{g/ml}$ ).....	62
Table 15. Number of eggs per adult for DBM across three different trials among different AgNPs concentration (0 $\mu\text{g/ml}$ , 4.32 $\mu\text{g/ml}$ and 8.64 $\mu\text{g/ml}$ ).....	62
Table 16. Mean ( $\pm\text{SE}$ ) data for different variables of Diamondback Moth across two different trials.....	71

## LIST OF FIGURES

Figure 1. DBM larvae exposed to SWCNT artificial feed.....	64
Figure 2. Scatter plot of growth/larva ( $\mu\text{g}$ ) for consumption/larva ( $\mu\text{g}$ ) of DBM exposed to different SWCNT concentration. ....	65
Figure 3. DBM larvae exposed to AgNP artificial .....	68
Figure 4. Scatter plot of growth/larva ( $\mu\text{g}$ ) for consumption/larva ( $\mu\text{g}$ ) of DBM exposed to different AgNPs concentration.....	69
Figure 5. CYP6BG1 gene expression level for different NPs concentration on DBM.....	70

## INTRODUCTION

### 1.1. Introduction to Nanotechnology and Engineered Nanoparticles

Nanoparticles that result from human activities (e.g., combustion) or are engineered for consumer products and new technologies are probably encountered by all organisms (Buzea *et al.* 2007). According to the American Society for Testing Materials, and the Scientific Committee on Emerging and Newly-Identified Health Risks, engineered nanoparticles (ENPs) can be defined as manufactured materials having at least two dimensions between 1–100 nm. ENPs can be categorized into different classes; for example, metals, metal oxides, non-metals, polymer based, functionalized (Klaine *et al.*, 2008). ENPs can exhibit many novel properties and reactivity because they have high surface to volume ratio compared to other larger sized materials with similar chemical composition (Hochella *et al.*, 2008; Auffan *et al.*, 2009). ENPs have novel traits in terms of their form and function, unique physical and chemical properties, design, potentially complicated interactions with biological and environmental agents, potential bio-persistence in organisms and feed chains, quick dispensability, bioaccumulation, penetrability through tissue, and irreversible biochemical activities.

ENPs are increasingly used in a wide range of products and technologies; from electronic devices to renewable energy to cosmetics and medicine (Christian, 2009; Fabrega *et al.*, 2011). According to, consumer product inventories (CPI) there were 653 products in 2007 containing ENPs but there were 1202 consumer products in 2014 containing NPs (Vance *et al.* 2015). This number is expected to increase significantly

over time. However, there are no data that estimate ENP concentrations or distribution in the environment (Klaine *et al.*, 2008).

Because of their novel properties (size, shape, specific surface area, size distribution, chemical composition, and surface structure), some ENPs are thought to be potentially toxic (Sahu and Casciano, 2009; Sharifi *et al.*, 2012). Several researchers reported that nanoparticles are more toxic than their counterpart microparticles (Shi *et al.*, 2001; Hoet *et al.*, 2004; Oberdorster, 2004; Yang and Watts, 2005; Borm *et al.*, 2006; Hund-Rinke and Simon, 2006; Powell and Kanarek, 2006). Since ENPs do not have any natural analog, it is difficult to forecast their fate, transport, reactivity, and toxicity in the environmental systems. Therefore, there are concerns about their potential negative effects when released into the environment (Maynard *et al.*, 2011; Colvin, 2003; Lowry *et al.*, 2012).

## **1.2. Carbon Nanotubes**

Carbon nanotubes (CNTs) (Iijima 1991) and single walled carbon nanotubes (SWCNTs) (Iijima and Ichihashi, 1993) were developed in 1991 and 1993 respectively. A CPI report (2013) showed that CNTs are the 3rd most common ENP after silver and titanium. CNTs are an allotrope of carbon, and there are three main types of CNTs: single-walled CNTs (SWCNTs), double-walled CNTs (DWCNTs) and multi-walled CNTs (MWCNTs). These types can be defined by their structure and diameter. The synthesis methods for CNTs are mainly carbon-arc discharge, carbon laser ablation, or chemical vapor deposition (CVD). CVD and arc discharge are the most commonly used

method to produce SWCNTs (Cheng *et al.*, 2007). Raw CNTs can be purified and functionalized, which may alter their toxicity (Sun *et al.*, 2002; Bianco *et al.*, 2005).

SWCNTs are developed from a single graphite sheet rolled into a cylindrical tube (Fako and Furgeson, 2009) and have a diameter of 0.7 to 3.0 nm, with the length can be hundreds of micrometers. SWCNTs have extraordinary electrical, mechanical, thermal, optical, and chemical properties (Dresselhaus *et al.*, 2001; Baughman, *et al.* 2002; Gracia-Espino *et al.*, 2010). They can have metallic, semi-conductive, and superconductive properties and high tensile strength (Avouris, 2002; Baughman, *et al.* 2002). SWCNTs can be found in field-effect transistors (Bradley *et al.*, 2003; Snow *et al.*, 2003; Meitl *et al.*, 2004; Zhou *et al.*, 2004), chemical sensors (Maklin *et al.*, 2007), nano-electronic devices (Rueckes *et al.*, 2000; Bachtold *et al.*, 2001; Avouris, 2002), hydrogen storage cells (Dillon *et al.*, 1997; Liu *et al.*, 1999; Kayiran *et al.*, 2004), sorbents for toxic chemicals (Long and Yang, 2001a; Long and Yang, 2001b; Lacerda *et al.*, 2006), *in situ* sensing (Kruss *et al.*, 2013), imaging (Robinson *et al.*, 2012), and for thermal ablation (Kosuge *et al.*, 2012). They have a very high surface area which allows for extensive molecular loading and multi-functionalization, as well as efficient internalization into a cell in a large number (Kostarelos *et al.*, 2007; Boyer *et al.*, 2016). They can also transport proteins, oligonucleotides, long nucleotide chains, (Kam *et al.*, 2006) and drugs to specific sites in organisms (Bianco *et al.*, 2005).

Raw SWCNTs are mainly hydrophobic, whereas purified SWCNTs are mainly hydrophilic because of the functional groups on their surfaces (Sun *et al.*, 2002). There is controversy about whether purification of raw SWCNT reduces (Sayes *et al.*, 2006) or increases (Tian *et al.* 2006) their toxicity; and the effect of functionalized SWCNT on

different animals during their early development is still unknown (Philbrook *et al.*, 2011). Various functionalization and dispersion methods, as well as their differential fates, could increase or decrease their toxicity (Kennedy *et al.*, 2009).

Concerns about potential risks of organisms exposed to SWCNTs are also paralleled with their increasing use, disposal, and diffusion into the environment. At present, studies for SWCNTs toxicity are limited (Shvedova *et al.*, 2008; Donaldson *et al.*, 2010; Johnston *et al.*, 2010; Castranova and Mercer, 2012; Ong *et al.*, 2016b). To date, there have been several studies published that examine the toxicity of SWCNTs on vertebrates. On the other hand, very few studies have been conducted on arthropods, yet, arthropods will potentially be exposed to SWCNTs in the environment. Concerns about environmental risks of SWCNTs and considerations about the research on toxicity testing have already been expressed in several reports and review papers (Petersen and Henry, 2011; Du *et al.*, 2013). Proactive research for clear understanding about the toxicity of SWCNTs toxicity is increasingly important with their production, use and disposal in ever larger quantities.

### **1.3. Silver Nanoparticles**

Another class of engineered nanoparticle that are becoming increasingly common are Ag nanoparticles (AgNPs). There is interest in their use for antimicrobial (Lee *et al.*, 2007a; Demir *et al.*, 2011) and anti-inflammatory (Bar-Ilan *et al.*, 2009) properties. In addition, AgNPs are effective against multidrug resistance strains of bacteria such as methicillin-resistant *Staphylococcus aureus*, *Pseudomonas aeruginosa* (Palanisamy *et al.*, 2014), ampicillin-resistant *Escherichia coli* O157:H7, and erythromycin-resistant

*staphylococcus pyogenes* (Shahverdi *et al.*, 2007). Consequently, AgNPs have advantages over other antibiotics (Yamanaka *et al.*, 2005; Lara *et al.*, 2011).

Because of their properties, they are also increasingly incorporated into consumer products such as feed packaging (Edwards-Jones, 2009), deodorants, clothing materials, bandages (Chen and Schluesener, 2008), burn treatments (silver sulfadiazine), socks, soaps and detergents, water and air filters, washing machines, wet wipes, bedding, coating on surgical instruments and medical and industrial textiles (Buzea *et al.*, 2007; Chen *et al.*, 2007; Kumari *et al.*, 2010; Liu *et al.*, 2010). They are also used in optical microscopy, surface-enhanced Raman spectroscopy (SERS), nanodevices for biological sensing (Sun and Xia, 2003). AgNPs are part of HIV, ovulation and pregnancy tests (Wagner *et al.*, 2006), and used in cancer and tumor detection (Ong *et al.*, 2016a). AgNPs can be infused into synthetic tissue adhesives in ophthalmic applications, as they have higher antibacterial properties and greater mechanical strength (Yee *et al.*, 2015).

With the use of high quantities of AgNPs, the accumulation in the environment and the exposure of living tissue of AgNPs are increasing (Chen and Schluesener, 2008). There is little information about how Ag from AgNP work inside the cell (Armstrong *et al.*, 2013). Some researcher believes that the lethal properties of AgNP to microbial cells are also responsible for their toxicity to eukaryotic cells (Buzea *et al.*, 2007). There is some evidence that particle size or surface area (Oberdorster *et al.*, 2005; Risom *et al.*, 2005; Nel *et al.*, 2006; Oberdorster *et al.*, 2007; Rogers *et al.*, 2007; Jiang *et al.*, 2008) is mainly responsible for AgNP toxicity, as Ag<sup>+</sup> released from the NP surface after oxidation could enter into the body and interact with biological molecules (Moore, 2006; Lin *et al.*, 2010; Park *et al.*, 2010). Several researchers found that ionic Ag is highly toxic to

bacteria (Liau *et al.*, 1997), phytoplankton (Ratte, 1999), invertebrates, and fish (Croteau *et al.*, 2011). It is also known that, AgNPs interact with proteins (in particular with thiol groups) and promote denaturation of proteins (Johnston *et al.*, 2010). Ag also interacts with proteins associated with cellular copper homeostasis (Winder and Harris, 1991). Surface coatings, functionalization, and stabilization could also influence their toxicity and biocompatibility (Bar-Ilan *et al.*, 2009). For example, Posgai *et al.*, (2011) concluded that, smaller and uncoated particles were more toxic than larger and coated particles respectively.

#### **1.4. Neutral Effects of SWCNT on Animals**

There are a number of studies examining SWCNTs that have not found any measurable impact on animals. Lung tissue in rats exposed to 0.03 or 0.13 mg/m<sup>3</sup> SWCNTs in the air was not negatively affected (Morimoto *et al.*, 2012). Rats injected with SWCNTs did not show any abnormality in cardiac function (Joviano-Santos *et al.* 2014). Mice aspirated with SWCNTs at 40 µg were similarly not negatively affected (Kagan *et al.*, 2010). Finally, Philbrook *et al.* (2011) reported that there were no effects on litter sizes, maternal weight, fetal lengths, weight, and viability in pregnant mice exposed to 10 mg/kg SWCNTs with >90% purity and functionalized with a hydroxyl (–OH) group.

SWCNT contamination in Rainbow trout (*Oncorhynchus mykiss*) blood (in red and white blood cell counts), haematocrit, whole blood hemoglobin did not cause negative effects, and; tissue metal levels and ATPase activity in brain were also unaffected (Smith *et al.*, 2007). Fraser *et al.* (2011) reported no significant impact of 500



mg/kg SWCNTs on Rainbow trout mortality, consumption, growth, plasma protein, or ATPase activity in the gill, intestine, or brain. Cheng *et al.* (2007) reported no significant negative effects of 240 mg/L SWCNT solution on zebrafish on head trunk, body length, cell death, regulatory factors (myogenic, notochords and primary sensory neurons), pigment development, main blood vessel formation, or hatching success and survival rate. Templeton *et al.* (2006) found no negative effects on developmental, fertilization success, life-cycle mortality and growth rate in estuarine copepods *Amphiascus tenuiremis* exposed to 10 mg/L purified SWCNTs. *Daphnia magna* (48 h of exposure) and medaka fish *Oryzias latipes* (96 h of exposure) exposed to raw SWCNTs (upto 100 mg/L) did not show any mortality and immobility (Sohn *et al.*, 2014).

The number of SWCNT toxicity studies conducted on insects is very limited. *Drosophila melanogaster* supplemented with SWCNT feed (1000 µg/g) did not show any detectable toxicity on egg to adult survivorship, rather SWCNTs were sequestered in tissue (Liu *et al.*, 2009). No significant effect was observed on *D. melanogaster* fed on ~10 ppm of disaggregated SWCNT on their growth, viability, fertility, but SWCNTs was incorporated into tissues of the larvae (Leeuw *et al.*, 2007). Lee *et al.*, (2015) found that a 100 µg/ml SWNT had no effect on larval viability, lifespan, fecundity, pupal viability, physical activity of female flies and resistance to starvation stress on *D. melanogaster* larvae fed with carbon nanofiber, CNF. *D. melanogaster* 0.5%, w/v SWCNTs with >90% purity and functionalized with a hydroxyl (–OH) group showed no significant effect on fecundity, egg viability, developmental stage, and histology (Philbrook *et al.*, 2011).

### 1.5. Negative Effects of SWCNT on Animals

Manna *et al.* (2005) found that human keratinocyte cells exposed to 10  $\mu\text{g/ml}$  SWCNTs exhibited increased oxidative stress and cell proliferation inhibition. Cui *et al.* (2005) reported that, 25  $\mu\text{g/ml}$  SWCNTs decreased tyrosinase activity (responsible for pigment development) in human embryonic kidney cells (HEK293 cells). Choi *et al.* (2009) reported increased inflammatory responses, and elevated oxidative stress and cell membrane damage and Kisin *et al.* (2007) reported reduction of viability and induction of DNA damage in lung fibroblast (V79) due to SWCNT exposure. SWCNTs caused bundle formation of actin filaments as well as impairment in proliferative activity (Holt *et al.* 2010), induction of DNA damage due to incorporation into centrosome structure (Sargent *et al.* 2009), formation of stable protein complexes and disruption of protein structure (Zuo *et al.*, 2010), and damaging of ion channel function due to changes in proteins (Park *et al.*, 2003).

Legramante *et al.* (2009) reported decreased heart rate in rats due to SWCNTs. Mice intratracheally instilled with 0, 0.1, or 0.5 mg of SWCNTs for 7 days induced dose dependent epithelioid granulomas and interstitial inflammation (Lam *et al.*, 2004). Mice that inhaled 5  $\text{mg/m}^3$  SWCNTs produced elevated inflammatory responses, collagen deposition, oxidative stress, and fibrosis in the lungs (Shvedova *et al.*, 2008). Cardiopulmonary toxicity was reported for mice exposed to aspirated 10  $\mu\text{g}$  or 40  $\mu\text{g}$  SWCNTs (Tong *et al.*, 2009). Teegarden *et al.* (2011) reported that mice aspirated with 40  $\mu\text{g}$  SWCNTs twice a week produced severe inflammatory and fibrotic responses. Shvedova *et al.* (2014) found that mice that inhaled 5  $\text{mg/m}^3$  pure SWCNTs showed histopathological changes in the lungs and tracheobronchial lymph. Philbrook *et al.*

(2011) reported pregnant mice exposed to 10 mg/kg SWCNTs with >90% purity and functionalized with a hydroxyl (–OH) group showed higher percentage of resorptions, gross morphological defects, skeletal abnormalities, aberrations in tail structure, defects in cervical vertebrae and variable ossification of sternbrae. SWCNTs induced dose dependent cytotoxicity in alveolar macrophages of guinea pig (Jia *et al.*, 2005).

Smith *et al.* (2007) reported dose dependent effects in ventilation rate, gill pathologies (oedema, altered mucocytes, hyperplasia), mucus secretion and increased ATPase activity of gills and intestine in Rainbow trout (*O. mykiss*) exposed to SWCNTs. Rainbow trout supplemented with 500 mg/kg SWCNTs diet showed significant elevation in brain TBARS (an indication of lipid peroxidation) (Fraser *et al.*, 2011). SWCNTs caused hatching delay in zebrafish embryos exposed to 120, 240, and 360 mg/L with no obvious dose response (Cheng *et al.*, 2007).

Two freshwater microalgae (*Raphidocelis subcapitata* and *Chlorella vulgaris*) showed reduced growth rate and biomass exposed to raw SWCNTs (upto 46.10 mg/L) (Sohn *et al.*, 2014). SWCNTs were reported to effect ingestion and digestion of bacteria (Ghafari *et al.*, 2008), induction of toxic effects on the development of *Escherichia coli* (*E. coli*) biofilm (Rodrigues and Elimelech, 2010) and bacterial cell death due to SWCNTs aggregation (Kang *et al.* 2007).

Petersen *et al.* (2008) reported the accumulation of C-labeled SWCNT in the gut of *Lumbriculus variegatus*, 80% of SWCNTs were purged after 3 days. Mwangi *et al.* (2012), reported that 1 g/L SWCNT reduced the survival rate of an amphipod (*Hyaella azteca*), a midge (*Chironomus dilutus*), and an oligochaete (*Lumbriculus variegatus*). They also reported accumulation of SWCNTs on their gut. 10 mg/L complex as prepared

SWCNTs slowed development, reduced fertilization success and increased mortality of estuarine copepods *Amphiascus tenuiremis* (Templeton *et al.*, 2006). The arthropod *D. magna* showed reduced immobilization and increased mortality when exposed to 100 mg/L SWCNTs (purity > 60%) (Zhu *et al.*, 2009). Roberts *et al.* (2007) reported that lipid-coated SWCNTs accumulated in the gut *D. magna*, and the animals suffered acute toxicity (reduced survival rate and increased mortality) as a result of exposure at levels up to 2.5 mg/L.

There is very little information published, however, on the effects of CNTs on insects. SWCNT reduced climbing activity, overwhelmed the natural grooming mechanism, impaired locomotor function, and increased mortality rate in *D. melanogaster* (Liu *et al.*, 2009). Lee *et al.* (2015) reported that *D. melanogaster* larvae fed on 1000 µg/ml CNF had reduced larval viability, lifespan and fecundity. Male flies also had reduced physical activity. They also found that *Drosophila* larvae developed resistance against starvation stress, generated increased number of crystal cells (A type of blood cell produced when flies face any immune challenge), and reactive oxygen species.

### **1.6. Neutral Effect of AgNPs on Animals**

Sprague-Dawley Rats that inhaled AgNPs ( $1.32 \times 10^6$  particles/cm<sup>3</sup>, 61 µg/m<sup>3</sup>) for 28 days had no changes feed consumption, body weight, organ weight, hematology or blood biochemical values. However, AgNPs were detected in liver and heart tissue in a dose dependent manner (Ji *et al.*, 2007). Feed and water consumption, and female body weight of F344 rats fed was not affected by 56 nm AgNPs (up to 500 mg/Kg) in their feed (Kim *et al.*, 2010).

Lim *et al.* (2012) found no adverse effect in *C. elegans* on pmk-1 (km25) mutant in case of ROS formation and HIF-1 and GST activation and reproduction rate after exposure to AgNPs. Zhao and Wang (2011) found that, 500 µg/ml AgNPs are not acutely toxic to *D. magna*. They also reported no significant toxic effect of AgNPs on mortality, but very high amount of accumulated AgNP in gut linings. Kim *et al.* (2011) reported that, AgNPs suspensions excluding Ag<sup>+</sup> were not acutely toxic to *D. magna*.

Silver-Key *et al.* (2011) reported that 5%/ ~9000 ppm AgNPs did not affect *D. melanogaster* hatching rates and that 50 mg/L of AgNPs did not affect adult survival, metamorphosis, and lifespan of *D. melanogaster* (Armstrong *et al.*, 2013).

### **1.7. Negative Effects of AgNPs on Animals**

There are a handful of studies that have found AgNPs toxicity to vertebrates. Haase *et al.* (2011) reported AgNPs strong cytotoxicity and cell death on human macrophages (monocytic leukemia cell line THP-1) in dose and time dependent manner. They also concluded that the smaller AgNPs are more toxic than larger ones. AgNPs caused a significantly higher amount of free radical generation and glutathione content reduction in BRL 3A rat liver cells (Hussain *et al.*, 2005). Ahamed *et al.* (2008) reported DNA damage and apoptosis in mouse embryonic stem cells and fibroblasts. Kim *et al.* (2008) found significant dose-dependent changes in rats for alkaline phosphatase activity, cholesterol level and liver function. Tang *et al.* (2008) reported accumulation of AgNPs into rat brain after 62.8 mg/kg AgNP injection and subsequent neuronal degeneration and necrosis. 56 nm AgNPs (up to 500 mg/Kg) fed to F344 rats resulted significant decrease in male body weight, slight liver damage and higher incidence of bile-duct hyperplasia

(Kim *et al.*, 2010). Rahman *et al.* (2009) found that 25 nm sized AgNPs (up to 1000 mg/kg AgNP) induced neurotoxicity in male C57BL/6N mice by altering gene expression, generating oxidative stress, and inducing apoptosis. AgNPs were found to inhibit the proliferation of mouse spermatogonial stem cells through blocking GDNF/Fyn kinase signaling (Braydich-Stolle *et al.*, 2010). Significant negative effects of AgNPs in rates were observed for spermatogenic cell number (Miresmaeili *et al.*, 2013), sperm abnormalities (Mathias *et al.*, 2014), and spermatocyte development (Han *et al.*, 2016).

Citrate capped AgNPs (0.04–0.71 nM) transported into the zebrafish embryo induced deformities and finally death (Lee *et al.*, 2007b). Bar-Ilan *et al.* (2009) reported that zebrafish exposed to 250  $\mu$ M AgNPs had decreased survival rates and suffered the following abnormalities: stunted growth, opaque and nondepleted yolk, small head, jaw, snout, tail and circulatory malformations, and body degradation. Choi *et al.* (2010) reported that AgNPs at a concentration of 250 mg/L AgNPs was lethal to zebrafish. Choi *et al.* (2010) also found induction of metal-sensitive metallothionein 2 (MT2) mRNA in liver tissue and reduced levels of oxyradical-scavenging enzymes catalase and glutathione peroxidase 1a mRNAs. They also found such cellular alterations as disruption of hepatic cell cords and apoptotic changes and increased level of malondialdehyde (a byproduct of cellular lipid peroxidation) and total glutathione. They also documented increased DNA damage and upregulation of the *Bax*, *Noxa*, and *p21* genes. Yoon *et al.* (2007) demonstrated that AgNPs were toxic to bacteria.

Ali *et al.* (2014) reported lethal (48.10  $\mu$ g/L) and sublethal (36  $\mu$ g/L) toxicity of AgNP in the fresh water snail *Lymnaea luteola* L.; at sublethal concentration, AgNPs caused higher lipid peroxidation, catalase activity in the digestive gland, DNA damage,

and reduced glutathione level, and increases in glutathione peroxidase and glutathione-S-transferase activities. Meyer *et al.* (2010) reported AgNPs to be toxic to *C. elegans*. Kim *et al.* (2012) found reduced survival and reproduction, generation of severe epidemic edema and burst (even though there were no evidence of AgNP intake) in *C. elegans* exposed to citrate-coated AgNPs (up to 100 mg/L). *C. elegans* increased ROS formation, expression of PMK-1 p38MAPK and hypoxia-inducible factor (HIF-1), GST enzyme activity and decreased reproduction in wildtype (N2) after exposure to AgNPs (Lim *et al.*, 2012). Zhao and Wang (2011) reported chronic toxicity in *D. magna* exposed to AgNPs. Poynton *et al.* (2012) reported citrate/PVP coated AgNPs toxicity on *D. magna*. They found disrupted major biological process by AgNPs (i.e. protein metabolism, signal transduction); PVP coated AgNPs induced metal responsive and DNA damage repair genes. Citrate coated AgNPs were more toxic than same sized PVP coated AgNPs.

*D. melanogaster* is the only insect that has been experimentally exposed to AgNPs to examine toxicity. Ahamed *et al.* (2010) reported that *D. melanogaster* fed with 100 µg/ml AgNPs responded with the following changes: increased expression of Hsp70, p53 and p38 protein (due to increased DNA damage), induction of oxidative stress and higher activities of caspase-3 and caspase-9 (apoptosis). Demir *et al.* (2011) reported that AgNPs (10 mM) induced genotoxicity of *D. melanogaster* larvae. Posgai *et al.* (2011) reported negative, dose dependent (up to 200 µg/ml) effects of AgNP on *D. melanogaster* survivorship, pupation, mating success, and abnormalities in cuticular and melanization development. Silver-Key *et al.* (2011) reported impaired larval developmental progression, reduced body pigmentation, shortened life span, abnormal climbing behavior and stress induction in *D. melanogaster* larvae fed with 5% / ~9000 ppm AgNPs. *D.*

*melanogaster* showed demelanized adult cuticle, reduced progenies, verticle movement, tyrosinase and Cu-Zn superoxide dismutase activity when feed was supplemented with 50 mg/L of AgNPs (Armstrong *et al.*, 2013). Ong *et al.* (2016a) reported accumulated AgNPs in *D. melanogaster* gut, reduced viability, slower developmental process, reduced fecundity of males and increased level of ROS at the apical tip of the testis when fed with 5 mg/L AgNPs.

### **1.8. Measurement of Gene Expression of CYP6BG1**

The cytochrome (CYP) P450 monooxygenases (p450s) are an abundant gene superfamily of heme-thiolate proteins, and this group of enzymes is found in almost all living organisms (Werck-Reichhart and Feyereisen, 2000). CYP genes can be categorized into 4 major clans; CYP2, CYP3, CYP4 and mitochondrial (Nelson, 1998). They are involved in the first step of drug metabolism, in detoxification of numerous xenobiotics and endogenous substances, and are essential for proceeding to second step of detoxification (Pelkonen *et al.* 1998; Martignoni *et al.* 2006; Fröhlich *et al.*, 2010).

In an *in vitro* study Sereemaspun *et al.* (2008) reported inhibition of CYP1A2, CYP2C19, and CYP3A4 in heterologously expressed human p450s in insect cell membrane exposed to 15 nm sized AgNPs. In Rainbow trout, 10 nm sized AgNPs caused CYP1A2 induction in gill tissue (Scown *et al.*, 2010). Kulthong *et al.* (2012) found no significant effect of orally administered AgNPs (~180 nm diameter; up to 1000 mg/kg) on CYP activities in vivo in Sprague-Dawley rat, but reported inhibition of CYP2C and CYP2D activities *in vitro*. SWCNTs inhibited CYP3A4BR activity in a dose-dependent



manner by choking the exit channel of substrate/products through a complex mechanism in Bactosomes (El-Sayed *et al.*, 2016).

The CYP3 clan is the largest clan (Cui *et al.*, 2017) incorporating with CYP6 and CYP9 gene families. They are found among insect p450 genes in large clusters (Feyereisen, 2006). Gene families from this clan play very important roles in insects via inactivation and metabolism of xenobiotic compounds such as insecticides and pesticides (Scott, 1999; Feyereisen, 2005; Bernhardt, 2006; Iga and Kataoka, 2012; lin *et al.*, 2013). Genes from this clan are referred to as “environmental response genes” (Berenbaum, 2002).

Resistance to toxic chemicals in insects are often associated with one or more detoxifying genes; e.g., p450s, esterases and glutathione S-transferases (Matambo *et al.* 2010; Stevenson *et al.* 2011; Niu *et al.* 2011; Martinez-Paz *et al.* 2012). CYP6B enzymes are believed to be mostly responsible to insecticides in caterpillars (Cohen *et al.*, 1992; Berenbaum *et al.*, 1996). In several insects, a large number of CYP6 genes have been identified, which are associated with toxic chemical resistance. For example, CYP6A1 (Carino *et al.*, 1992; Carino *et al.*, 1994), CYP6D1 (Tomita *et al.*, 1995; Liu and Scott, 1998) and CYP6A12 (Guzov *et al.*, 1998) in housefly; CYP6CM1 (Karunker, *et al.*, 2008) in whitefly; CYP6CY3 (Puinean, *et al.*, 2010) in peach aphid; CYP6B3, CYP6B4, CYP6B5 (Scott and Wen, 2001) in butterfly *Papilio polyxenes*; CYP6BQ9 (Zhu *et al.*, 2010; Zhu *et al.*, 2013) in red flour beetle; CYP6BQ23 (Zimmer *et al.*, 2014) in pollen beetle; CYP6ER1 and CYP6AY1 (Bass *et al.*, 2011; Ding *et al.*, 2013; Bao *et al.*, 2016) in brown planthopper; CYP6G1 (Daborn *et al.*, 2001; 2008; Hoi *et al.*, 2014) in *D. melanogaster*; CYP6AB11 (Niu *et al.*, 2011) in *Amyelois transitella*; CYP9G2 (Shen *et*

*al.*, 2004) and CYP6BF1 (Li *et al.*, 2005) in diamondback moth (DBM). More than half of CYP P450 genes were upregulated in the resistance strain of Colorado potato beetle against imidacloprid pesticide (Zhu *et al.* 2016). Bautista *et al.* (2007) reported that, permethrin resistance at the fourth instar stage of DBM larvae was associated with CYP6BG1 overexpression in resistant strains and inducible in their susceptible counterpart. Bautista *et al.* (2009) confirmed that CYP6BG1 overexpression was due to increased metabolism for permethrin detoxification through RNA interference mediated gene silencing (RNAi).

Only few studies examined whether the CYP p450 gene is expressed differentially in animals exposed to, presumably, toxic concentrations of ENPs. El-Sayed *et al.* (2016) examined the effect of carboxylated SWCNT on animal CYP activity. Fröhlich *et al.* (2010), Lamb *et al.* (2010) and Warisnoicharoen *et al.* (2011) examined the effects of AgNPs on CYP activity.

## **1.9. Research Goals and Questions**

I examined the effects of these ENPs on DBM because it is considered a model pest. It is a major pest on cruciferous crops (Talekar and Shelton, 1993) and can migrate and reproduce very quickly (Yu *et al.*, 2015). It is remarkably resistant to insecticidal toxins (Sun *et al.*, 1986; Sun, 1992; Talekar and Shelton, 1993; Scott and Wen, 2001; Furlong *et al.*, 2013), and toxicity to ENPs would indicate that many insects might be negatively affected. For example, DMB was the first pest that became resistant to DDT (Ankersmit, 1935; Jhonson, 1953). In almost all countries they have evolved resistance

against agricultural synthetic insecticides (Talekar *et al.*, 1990), even the bio-insecticides based on *Bacillus thurengiensis* spores (Tabashnik *et al.*, 1997).

The goal of my research was to examine if SWCNTs or AgNPs are toxic to an insect herbivore, the DMB. The first question I addressed was whether these two ENPs were a deterrent to DBM feeding. I hypothesized that these ENPs do not deter feeding as the larvae consumed ENP feed. The second question I addressed was whether these ENPs had an effect on the amount of feed DMB consumed? I then examined if consumption of ENPs affected growth. For example, if they consume the same amount of control feed and feed with ENPs, will the larvae grow more or less? I then related ENPs consumption and growth rate to survival rates, pupation rates and fertility (number of eggs laid). Finally, my research examined if there was any evidence for detoxification of the ENPs? Specifically, were p450 genes upregulated or downregulated?

## MATERIALS AND METHODS

### 2.1. Single Walled Carbon Nanotube Properties

SWCNTs (COOH-CNT) produced by the catalytic vapor deposition (CVD) process were purchased from NanoLab Inc. (Waltham, MA, USA) in powdered form. For this study, they were dispersed in 18M $\Omega$  deionized water to make the desired concentration (290 mg/L or 292 mg/L or 305 mg/L) with more than 95% purity and pH of 6.5 -7.5. Initially, 400 mg of SWCNT material were added to 1L of 18M $\Omega$  deionized water. A probe sonicator was used for dispersion (twice for 30 min with a 15 minutes' rest in between) followed by ultracentrifugation at 25,000g for 30 min. The supernatant collected from the centrifuge tubes and ultracentrifugation was repeated at 25,000g for 30 minutes. During this process, the concentration decreases due to the removal of amorphous carbon and unfunctionalized carbon material and finally yield an approximate 300 mg/L desired concentration. The SWCNTs have an approximate diameter of 1.5 nm, length of 1-5  $\mu$ m and surface area of 1020.48 m<sup>2</sup>/g.

### 2.2. AgNPs Properties

AgNPs (20 nm PELCO® citrate NanoXact™ AgNPs) were commercially obtained from Ted Pella Inc. (Redding, CA, USA). Their diameter was  $18.5 \pm 3.4$  nm and their hydrodynamic diameter was 28 nm with 29.0 m<sup>2</sup>/g surface area (TEM). The concentration of the AgNP suspension was 0.021 mg/ml, with a particle concentration of  $6.0E + 11$  particle/ml. The AgNP has -43 mV zeta potential and 396 nm absorbance peak ( $\lambda_{\text{max}}$ ) with 3.50 max Optical Density/cm in a solution with 8.1 pH. The particle surface was sodium citrate and the aqueous carrier was 2 mM citrate. AgNPs were stored at 4°C

and away from light before use and they were used directly without any processing or change before use.

### **2.3. Artificial Feed Preparation**

The lab benches were sterilized and cleaned with 75% ethanol before the preparation of the experimental feed to avoid any infection to DBM larvae through feed with any natural microorganism. The artificial feed was a dry mix purchased from Southland Products Inc. (Lake Village, AR, USA). The mix was specifically formulated for DBM (*P. xylostella*). The recipe for 250 ml feed was as follows: 40.5 g dry mix and 1.75 ml raw linseed oil and 232.5 ml deionized boiling water. For SWCNT feed preparation, 1.75 ml raw linseed oil was added to 40.5 g dry mix in an Erlenmeyer flask. Then SWCNT solution was added in 7.45 ml, 14.89 ml, 29.79 ml, 59.58 ml or 119.2 ml volume to generate 8.64 µg/ml, 17.28 µg/ml, 34.56 µg/ml, 69.12 µg/ml and 138.24 µg/ml SWCNT feed respectively. The mixture was then combined with deionized boiling water (225.05 ml, 217.61 ml, 202.71 ml, 172.92 ml and 113.3 ml respectively) on a magnetic stirrer hot plate. After mixing the final suspension for 2-3 minutes, the resulting semi-liquid feed was poured into labeled Petri dishes and left in room temperature about 15-20 minutes for solidification. The solidified feed mixture was then partitioned using a small corer (1.3 cm diameter) and the remaining feed was stored in the refrigerator at 4°C.

To prepare 60 ml AgNP feed, 0.42 ml raw linseed oil was added to 9.72 g dry mix in a flask. Then AgNP solution was added in a volume of 12.34 ml and 24.69 ml to achieve a final concentration of 4.32 µg/ml and 8.64 µg/ml AgNP feed respectively. It was then combined with deionized boiling water (43.46 ml and 31.11 ml respectively).

Other steps were the same as SWCNT feed preparation. Nanomaterial free feed were used as control feed. There were also two other “control” arenas: 1) No larvae control (NLC), feed discs from all treatments without any larvae to determine any differences in how fast control and ENP feed discs dry out, 2) No feed control (NFC), starvation arenas with larvae but no feed to determine starvation rates.

#### **2.4. DBM Rearing**

Eggs of DBM were purchased commercially from Benzon Research Inc. (Carlisle, PA, USA). Eggs on aluminum foil were kept with artificial control feed discs in plastic feed boxes under diurnal cycle of 16 h light: 8 h dark to hatch and grow until they are 2<sup>nd</sup> instar larvae. The lab temperature was  $25 \pm 3^{\circ}\text{C}$  with  $65 \pm 5\%$  relative humidity. The DBM laboratory is licensed by the United States Department of Agriculture to rear and 3w: P26P-14-02726.

#### **2.5. Artificial Feed Assays**

Four artificial feed discs were placed in each petri plates except for the starvation arenas. There were six trials for SWCNT and two trials for AgNP. Details about the treatments and number of replicates are given in Table 1. From the artificial feed, the larvae were provided with 0.0 mg, 51.3 mg, 85.7 mg, 172.0 mg, 294.6 mg, 557.6 mg SWCNT and, 8.2 mg and 15.4 mg of AgNP.

Fifteen DBM larvae were placed into each Petri dish (treatment arena) except for NLC and allowed to consume the feed for about 72 hours (until pupation). Artificial feed

consumption by larvae and larval growth were determined by weighing feed discs and larval weight every 8-12 hours.

## **2.6. Measurement of Fecundity**

To collect the eggs, I used cabbage treated aluminum foil. Foil was cut into 10 cm X 4 cm pieces and straight parallel lines were drawn into the foil with a needle. Then they were dipped into freshly prepared cabbage juice. 26 grams of fresh cabbage were blended with 200 ml distilled water and then strained. The foil pieces were then soaked in the juice and left to dry. This was repeated at least 5 times and finally the foils were left to dry overnight. The foils were folded half (to 5 cm X 2 cm) and hung from the lid of the oviposition container, which were prepared from 5.5 oz. cups.

After the larvae went into pupation, they were transferred into oviposition containers. The containers were supplied with fresh artificial feed after adult emergence. Folded foils with appropriate leveling were hung from the lid to facilitate egg laying. Foils were changed after every 2 days and stored in a refrigerator at 4°C for about 4-6 days. Eggs were counted after 9/10 days of adult emergence.

## **2.7. Total RNA Extraction, Purification and Quantitation and cDNA Synthesis**

Fourth instar larvae (whole body) were collected and stored at -82°C. Five larvae were collected from each of the five replications of control, and the 138.24 µg/ml SWCNT treatment and the 4.32 µg/ml and 8.64 µg/ml AgNP treatment arenas. For total RNA extraction, the larvae were placed in liquid nitrogen and crushed using a porcelain mortar and pestle. All the equipment's were cleaned with 70% ethanol and RNase-Away (Molecular BioProducts Inc., San Diego, CA, USA) to eliminate contamination. The

larvae were then homogenized in 750 µl Trizol Reagent (life technologies, Carlsbad, CA, USA). Total RNA was precipitated with 500 µl isopropanol and resuspended in 50 µl DEPC treated water.

Extracted RNA was purified with Qiagen RNeasy® Mini Kit (Thermo Fisher Scientific, Eugene, Oregon, USA) following manufacturer's guidelines. I used Qubit® 3.0 Fluorometer and Qubit® RNA BR assay kits (Invitrogen, Eugene, Oregon, USA) to quantitate the RNA following the manufacturer's guidelines. The samples were then stored at -80°C until next use.

Three samples containing RNA at high concentration were selected from each treatment and first strand cDNA was synthesized with Qiagen QuantiTect® reverse transcription kit (Chatsworth, CA, USA) following the manufacturer's guidelines. I also performed reverse transcription using both oligo dT and random primers, but the QuantiTect® kit provided the highest yield of cDNA. All RNA samples were reverse transcribed simultaneously to avoid variations in cDNA. The cDNA was then amplified by regular PCR and confirmed with agarose gel electrophoresis. cDNA was stored at -20°C until next use.

## **2.8. Quantitative Real-Time PCR (qRT-PCR) Analysis**

Permethrin resistance gene CYP6BG,1 a member of the p450 gene family, was used as a target gene, and ribosomal protein L32 (RPL32) was used as a reference gene for qRT-PCR. The primer design for CYP6BG1 was reported previously by Bautista *et al.* (2007). Fu *et al.* (2013) and Gao *et al.* (2016) reported that RPL 32 is a reliable



housekeeping gene. I used the Integrated DNA Technologies website to design the forward and reverse primer for RPL 32 (Table 2).

The qPCR was performed with GoTaq<sup>®</sup> qPCR master mix (Promega Corporation, Madison, WI, USA) in an Mx3000P thermal cycler (Agilent Technologies, Inc. Santa Clara, CA, USA) with the help of MxPro qPCR software. 96 well EU thin-walled PCR plates (BPCTi inc., Durham, NC, USA) were used for the reaction. To determine PCR amplification efficiency, standard curves were generated for both target and reference gene using 10-fold serial dilutions. Thermal cycling profiles used in this study were: 95°C for 10 min, followed by 40 cycles of 95°C for 30 sec, 55°C for 60 sec, 72°C for 60 sec. A dissociation step cycle at 95°C for 60 sec, 50°C for 30 sec and 95°C for 30 sec was added as a final step to generate melting curves. The amplification reaction was done in three technical replicates for each biological replicate. No-template controls (NTC) were run for every sample to check for DNA contamination. The gene expression level was calculated based on cycle threshold (C<sub>t</sub>) value by using Pfaffl method (Pfaffl *et al.*, 2002).

## **2.9. Statistical analysis**

Data were analyzed using statistical software Minitab 17 (State College, PA; USA). I used a general linear model ANOVA to test for treatment effects on feed consumption, larval growth, % pupation, % survival, fecundity, and gene expression level. Data are presented as mean  $\pm$  standard error of mean. Tukey's test was performed for pairwise comparisons when main treatment effects in the ANOVA were statistically significant at  $p < 0.05$ . I started feeding trials using 8.64  $\mu\text{g/ml}$  SWCNT in artificial feed (Trial 1). In every subsequent trial, I doubled the concentration until I reached a concentration of 138.24  $\mu\text{g/ml}$  SWCNT, the highest concentration that I could

incorporate into the artificial feed. I used untransformed, natural log-transformed and Box-Cox transformed data because consumption and growth data are generally not normally distributed. However, there was no significant differences in the results. Consequently, I present untransformed data.

## RESULTS

### 3.1. Effect of SWCNTs on DBM

**3.1.1. Effect of SWCNTs on Feed Consumption.** There was no evidence that any concentration of SWCNT deterred feeding (Table 3). If the CNT deterred feeding than consumption of CNT feed would have always been less than consumption of control feed. There were no clear negative or positive effects of CNTs on consumption (Table 3). Larval feeding was not consistently affected by any concentration. For example, consumption of feed containing 8.64  $\mu\text{g/ml}$  SWCNT was not significantly different than control (Appendix parts 1 and 2); consumption of 17.28  $\mu\text{g/ml}$  SWCNT feed was not significantly different from the consumption of control feed (Appendix parts 1 and 2). However, 69.12  $\mu\text{g/ml}$  and 138.24  $\mu\text{g/ml}$  feed larvae consumed 10% and 4% more food than control food. Taken together, while there were some concentration and trials that suggested an apparent SWCNTs affects, there was no clear significant negative or positive effect detected (Appendix parts 1-6).

**3.1.2. Effect of SWCNTs on growth.** SWCNTs did not significantly affect DBM growth rate, except at the concentration of 138.24  $\mu\text{g/ml}$  (Table 4 and Appendix parts 1-6). For example, 8.64  $\mu\text{g/ml}$  and 17.28  $\mu\text{g/ml}$  feed larvae growth rate was same and lower (Table 4) compared to control, which cancelled each other's out. 34.56  $\mu\text{g/ml}$  feed larvae growth rate was same compared to control. But, 69.12  $\mu\text{g/ml}$  and 138.24  $\mu\text{g/ml}$  feed larvae growth rate was reduced almost 6% and 10% respectively. Since the larva became darkened in color (Figure 1) the SWCNTs may have not been metabolized but accumulated in their gut.

**3.1.3. Effect of SWCNTs on the rate of pupation.** SWCNTs did not had any measurable negative effect on the percentage of DBM pupation (Table 5). For the lowest SWCNT concentration of 8.64  $\mu\text{g/ml}$ , there was 100% pupation. However, there was a decreasing pupation rate with increasing SWCNT concentrations. For example: 17.28  $\mu\text{g/ml}$ , 34.56  $\mu\text{g/ml}$ , 69.12  $\mu\text{g/ml}$ , and 138.24  $\mu\text{g/ml}$  SWCNT fed larvae showed 3%, 4%, 6% and 4% reduction of pupation rate, respectively (Table 5). Even though the results were not significant, this reduced pupation rate suggests that there may be some level of SWCNT toxicity to DBMs.

**3.1.4. Effect of SWCNT on the rate of survival.** There was no significant effect of SWCNT on DBM survival rate (Table 6). For lower SWCNT concentrations i.e. 8.64  $\mu\text{g/ml}$  and 17.28  $\mu\text{g/ml}$  the survival was 100%. However, with the increasing SWCNT concentrations the survival rate decreased (Table 6). Survival rate reduced 2% for 34.56  $\mu\text{g/ml}$ , 6% for 69.12  $\mu\text{g/ml}$  and 3% for 138.24  $\mu\text{g/ml}$  SWCNT treatment. Even though the reduced survival rate was not significant, the observed trend warrants further investigation.

**3.1.5. Effect of SWCNT on feed conversion.** As the SWCNT fed larvae growth rate was same or less with same or higher consumption rate compared to control, we further investigated their feed conversion ratio; percentage of  $\mu\text{g}$  growth for  $\mu\text{g}$  feed consumption for each larva (Table 7). For example, 8.64  $\mu\text{g/ml}$ , 17.28  $\mu\text{g/ml}$ , 34.56  $\mu\text{g/ml}$ , 69.12  $\mu\text{g/ml}$  and 138.24  $\mu\text{g/ml}$  SWCNT fed larvae converted 10%, 3%, 6%, 16% and 12% less compared to control (Appendices 8-13).

**3.1.6. Effect of SWCNT on fecundity.** I investigated potential SWCNT effect on their fecundity for trial 4,5 and 6 (Table 8). All the SWCNT fed larvae showed reduced

fecundity relative to control fed larvae. Fecundity was reduced by almost 17% and 45% with elevated SWCNT concentration (69.12  $\mu\text{g/ml}$  and 138.24  $\mu\text{g/ml}$ , respectively) which is an indication of a sub-lethal toxic effect.

**3.1.7. Effect of SWCNTs Across All Data.** I combined and analyzed the entire data set for all the variables (Table 9). Consumption of feed containing SWCNTs was not significantly different than consumption of control feed. Growth was statistically significantly affected, even though the growth rate had an increasing tendency from 8.64  $\mu\text{g/ml}$  to 69.12  $\mu\text{g/ml}$ , at highest concentration (138.24  $\mu\text{g/ml}$ ) the growth rate decreased. For 50 hours, the larvae grew at a similar rate, after which their growth rate ended up at around same point (Appendices 22-27). Their pupation rate was not significantly different for individual trials. But the accumulated pupation rate is significantly different. Except for the lowest concentration (8.64  $\mu\text{g/ml}$ ), For all the SWCNTs concentrations (17.28  $\mu\text{g/ml}$ , 34.56  $\mu\text{g/ml}$ , 69.12  $\mu\text{g/ml}$ , and 138.24  $\mu\text{g/ml}$ ), DBM showed negatively correlated with reduced pupation rates, except for 8.64  $\mu\text{g/ml}$  SWCNT. I also examined survival rate, which was not statistically affected by SWCNTs. Fecundity (egg/adult) was negatively correlated statistically significantly affected, with the increasing with SWCNT concentration, fecundity was with an overall reduction of reduced by 45% in fecundity. This negative correlation was significant. Conversion rate was statistically significant, lower concentrations has lower conversion ratio compared to control, only our highest concentration 138.24  $\mu\text{g/ml}$  has higher conversion ratio than control. This might be insect's antioxidant defense response (Lee *et al.*, 2015)

The regression line for each SWCNT concentration showed that larva fed with 8.64  $\mu\text{g/ml}$  of SWCNT feed larva grew more as they consumed more feed. ate more, but

control larvae the growth rate of the control larvae was stable over time (Figure 2.a). Larvae fed with 17.28  $\mu\text{g/ml}$  of SWCNT feed larval growth did not increase with increasing amount of feeding (Figure 2.b), whereas, the control feed larvae grew less with increased feeding. Control larvae and larvae fed with 34.56  $\mu\text{g/ml}$  of SWCNT and control feed larvae had a reduced growth rate was reduced with increasing feeding (Figure 2.c). Larvae fed with 69.12  $\mu\text{g/ml}$  and 138.28  $\mu\text{g/ml}$  of SWCNT feed larvae grew parallelly with control feed larvae, they ate less and grew less (Figure 2.d and Figure 2.e).

### **3.2. Effect of AgNP on DBM**

There were two artificial feed assays to investigate the direct effect of AgNPs (4.32  $\mu\text{g/ml}$  and 8.64  $\mu\text{g/ml}$ ) on DBM. I investigated the effect of AgNPs on consumption, growth, survival rate, pupation rate, and fecundity. AgNPs negatively affected DBM consumption (Table 10). At the level of both 4.32  $\mu\text{g/ml}$  and 8.64  $\mu\text{g/ml}$  of AgNP, larvae ate less AgNP feed than control. DBM showed reduced growth rate for both AgNPs concentration, but the reduction was not significant (Table 11). However, demelanized and stunted larvae were observed during artificial feed assays (Figure 3). AgNP reduced pupation rate by 10% for the 8.64  $\mu\text{g/ml}$  of AgNPs treatment (Table 12). I could not measure an impact on survival rate (Table 13) at either concentration of AgNPs used. But in my experiments, I tested very low AgNP concentration. Conversion rate differences were not significant (Table 14). For both AgNP concentration (4.32  $\mu\text{g/ml}$  and 8.64  $\mu\text{g/ml}$ ), conversion ratio was both higher and lower (Appendix parts 19 and 18 respectively) compared to control. For AgNPs, fecundity was not statistically significant

(Table 15). In trial 1, both AgNP concentration increased fecundity, where as in trial 2, the fecundity remains almost the same.

For the combined analysis, the consumption and pupation rate were significantly reduced on AgNP-fed larvae (Table 16). Consumption reduced significantly for both concentrations, but pupation rate was significantly reduced for the 8.64 µg/ml treatment only. This suggests sublethal toxicity of AgNPs to DBM. Growth, survival rate, fecundity, and conversion rate remained the same with control treatment (Table 16). From the regression lines, there was no clear relationship for AgNP-feeding and growth (Figure 4).

### **3.3. Effect of ENPs on CYP6BG1 gene expression level**

I performed qRT-PCR, and calculated relative gene expression levels using the Pfaffl method to detect differences in the expression of the CYP6BG1 gene expression between treated and control DBM. The efficiency for reference and target gene was 97.5% and 83.8% (Appendix parts 28 and 29) respectively. The expression level of CYP6BG1 gene was higher for all the ENP treatments (Figure 5), but they were not significantly different from control DBM (Appendix 21).

## DISCUSSION

### 4.1 Effect of SWCNT on DBM

There was no dose dependent toxic effect of SWCNTs on DBM feeding. Fraser *et al.* (2011) also did not find any significant dose dependent feeding effect of SWCNTs on rainbow trout, even though they used very high concentration (500 mg/kg) and different sized SWCNTs (outside diameter = 1.1 nm, length = 5–30 nm). To my knowledge, my research is the first to examine feeding by a caterpillar on artificial feed containing various concentrations of nanomaterials. As the consumption rate of larvae fed on 69.12 µg/ml and 138.24 µg/ml SWCNT was higher than control, I am speculating that, the larvae didn't receive the same rate of nutrients from the SWCNT food compared to control and started compensatory feeding. Probably C molecule inhibiting the larvae from metabolizing the SWCNT food.

I did not find any significant effect of SWCNTs on DBM growth rate except at the highest concentration (138.24 µg/ml). My results are consistent with the findings of: Philbrook *et al.* (2011), who reported no effect of SWCNT treatment on maternal weight of pregnant mice for 10 mg/kg SWCNT with >90% purity and functionalized hydroxyl group. Fraser *et al.* (2011) found no effect of 500 mg/kg SWCNT on rainbow trout growth rate; Cheng *et al.* (2007) reported same body length of zebrafish for 240 mg/L SWCNT treatment; Templeton *et al.* (2006) reported same growth rate of copepods for purified or as prepared 10 mg/L SWCNT; and Leeuw *et al.* (2007) reported same growth rate of fruit fly for ~10 ppm SWCNT.

Several researchers have reported that SWCNTs can accumulate in organs and the body cavity of animals as diverse as insects and mammals. For example, Holt *et al.*



(2010) reported incorporation of SWCNTs into centromere structure of actin filaments; Kang *et al.* (2007) reported SWCNT aggregation into bacterial cells; Mwangi *et al.* (2012) found accumulated SWCNT into amphipod, midge, and oligochaete gut; Leeuw *et al.* (2007) found accumulated SWCNT on drosophila tissue; Petersen *et al.* (2008) reported accumulated SWCNT into *Lumbriculus variegatus* gut; Roberts *et al.* (2007) reported deposited SWCNT into daphnia gut. Even though SWCNTs may not be acutely toxic and may not hinder growth of animals, except at very high concentration, they are being transferred through the feed chain (Roberts *et al.*, 2007). SWCNTs can be chronically toxic to animals even in low concentrations (Smith *et al.*, 2007). They are suspected to be more toxic to mammals than insects, which suggests that there can be biomagnification.

I found that for the higher SWCNT concentrations I examined (34.56 µg/ml, 69.12 µg/ml, and 138.24 µg/ml) pupation rate decreased, albeit the effect was not significant. One possibility is that this is due to sample size. Experimental report from Lee *et al.* (2015) on fruit fly for CNF supports my result, even though they tested larger particle-size (100 nm diameter) and higher concentration (1000 µg/ml) of CNF compared to the one used here.

I found that only the highest concentration of SWCNT may have any impact on survival, although it was not possible to measure this effect in a significant manner. There are many previous studies that found similar results. For example: Philbrook *et al.* (2011), Fraser *et al.* (2011), Cheng *et al.* (2007), Templeton *et al.* (2006), Leeuw *et al.* (2007), Liu *et al.* (2009), Lee *et al.* (2015) and Shon *et al.* (2014). Some studies also reported decreased survival rate and reduced viability. For example, Kisin *et al.* (2007)

reported reduced survival rate in human lung fibroblast cell line; Mwangi *et al.* (2012) reported reduced survival rate of amphipod, midge, and oligochaete; and Templeton *et al.* (2006) found reduce viability of copepods. All these studies used very diverse SWCNTs (different size and concentration), which makes it impossible to infer any overarching conclusion from these studies.

As mentioned in the introduction, animals are probably not able to metabolize SWCNTs. However, the possibility of chronic toxicity exists, and future research examining predators eating DBM feed with or without SWCNTs is warranted. Also, the feeding trials lasted for about 60-70 hours: only in 3rd and 4th instars. It is possible that this exposure is too short and that these later two instar stages have higher resistance than previous stages.

In my experiments, I found that fecundity was reduced by almost 45% at the highest SWCNT concentration, which is an indication of sub-lethal toxic effects. There are several published reports, i.e. Cheng *et al.* (2007), Templeton *et al.* (2006) and Lee *et al.* (2015) which supports my findings.

From all the combined analysis, I can say that SWCNTs have sub-lethal toxicity on DBM growth, pupation rate and fecundity. Further experiments on SWCNTs chronic toxicity on DBM are warranted.

#### **4.2. Effect of AgNPs on DBM**

AgNPs are deterrent to DBM feeding, even at lowest tested concentration of 4.32 µg/ml. There are few studies on AgNPs feeding rate. There are numerous studies on AgNPs negative effect on growth of various animals: Kim *et al.*, (2010) reported reduced

body weight in rats for 500 mg/Kg AgNP; Bar-Ilan *et al.*, (2009) reported stunted growth on zebrafish for 250  $\mu$ M AgNP; and Zhao and Wang, (2011) reported reduced growth rate of *daphnia* for 5  $\mu$ g/ml AgNP. All these experiments tested higher AgNP concentration than I tested in my experiments. Furthermore, I observed deformed, demelanized larvae with stunted growth, which is also similar with other experiments. For example, Mathias *et al.* (2014), Bar-Ilan *et al.* (2009), Lee *et al.* (2007b), Armstrong *et al.* (2013), Silver-Key *et al.* (2011) and Posgai *et al.* (2011).

I found reduced pupation rate for 8.64  $\mu$ g/ml AgNP concentration. Posgai *et al.* (2011) also reported similar results but their AgNP concentration was much higher than mine. I did not find any AgNP effect on DBM's survival rate. Armstrong *et al.* (2013) also reported no significant effect of adult survival on *drosophila* for 50 mg/L; Zhao and Wang (2011), reported no mortality of *daphnia* for 500  $\mu$ g/ml. There was no significant effect of AgNP on DBM's fecundity. Silver Key *et al.* (2011) reported unaffected hatching rates were on *drosophila* for 5%/ ~9000 ppm.

#### **4.3. ENPs effect on CYP6BG1 gene expression level**

I found that CYP6BG1 gene expression was higher in DMB feed ENPs than those feeding on feed with no ENPs. Even though my results were statistically significant, further studies are warranted. Bautista *et al.* (2007, 2009) reported and confirmed upregulation of CYP6BG1 gene due to permethrin resistance in DBM. CYP P450 gene family have 36 to 180 genes in insect genome (Feyereisen, 2012; Zhou *et al.*, 2014), which makes it very hard to target one or few genes for gene expression assessment due to ENPs exposure.

In conclusion, my study provides new insights on the effect of functionalized pure (>95%) SWCNT on intact arthropods, relevant to ecotoxicity studies and environmental concerns. In my study, I did not find any acute toxicity as determined by measuring consumption, growth, % pupation and % survival rate. But I cannot exclude the subtle and sublethal toxic effects at different trials. My study provides strong suggestion for further study of SWCNT effect on insect fecundity. Also, SWCNT accumulation in insect body cavity should be further investigated for their biochemical and genomic toxicity.

There are many studies on AgNPs toxicity to animals and at higher concentration and they were identified as toxic in most cases. Even though, I tested very lower concentration, they showed toxicity for consumption and % pupation. Future studies with higher concentration is needed to verify their toxicity on arthropods.

My study on CYP6BG1 gene expression emphasize that, insects specially DBM have very strong immunity and CYP P450 family helps insects to metabolize these toxic ENPs. De novo expression profile analysis due to ENPs exposure to DBM would be very interesting study.

## REFERENCES

- Ahamed, M., Karns, M., Goodson, M., Rowe, J., Hussain, S. M., Schlager, J. J., Hong, Y., 2008. DNA damage response to different surface chemistry of silver nanoparticles in mammalian cells. *Toxicology and Applied Pharmacology*. 233 (3): 404–410.
- Ahamed, M., Posgai, R., Gorey, T. J., Nielsen, M., Hussain, S. M., Rowe, J. J., 2010. Silver nanoparticles induced heat shock protein 70, oxidative stress and apoptosis in *Drosophila melanogaster*. *Toxicology and Applied Pharmacology*. 242(3): 263–269.
- Ali, D., Yadav, P. G., Kumar, S., Ali, H., Alarifi, S., Harrath, A. H., 2014. Sensitivity of freshwater pulmonate snail *Lymnaea luteola* L., to silver nanoparticles. *Chemosphere*: 104, 134-140.
- American Society for Testing and Materials. 2006. Standard terminology relating to nanotechnology. E 2456-06. West Conshohocken, PA.
- Ankersmit, G.W., 1953. DDT resistance in *Plutella maculipennis* (Curt.) (Lepidoptera) in Java. *Bulletin of Entomological Research*. 44(3): 421-425.
- Armstrong, N., Ramamoorthy, M., Lyon, D., Jones, K., Duttaroy, A., 2013. Mechanism of silver nanoparticles action on insect pigmentation reveals intervention of copper homeostasis. *PLoS One*. 8(1): e53186.
- Auffan, M., Rose, J., Bottero, J. Y., Lowry, G. V., Jolivet, J. P., Wiesner, M. R., 2009. Towards a definition of inorganic nanoparticles from an environmental, health and safety perspective. *Nature Nanotechnology*. 4: 634–641.
- Avouris, P. 2002. Molecular electronics with carbon nanotubes. *Accounts of Chemical Research*. 35(12): 1026-1034.
- Bachtold, A., Hadley, P., Nakanishi, T., Dekker, C., 2001. Logic circuits with carbon nanotube transistors. *Science*. 294(5545): 1317- 1320.
- Bao, H., Gao, H., Zhang, Y., Fan, D., Fang, J., Liu, Z., 2016. The roles of CYP6AY1 and CYP6ER1 in imidacloprid resistance in the brown planthopper: Expression levels and detoxification efficiency. *Pesticide Biochemistry and Physiology*. 129: 70-74.
- Bar-Ilan, O., Albrecht, R. M., Fako, V. E., Furgeson, D. Y., 2009. Toxicity assessments of multi-sized gold and silver nanoparticles in zebrafish embryos. *Small*. 5: 1897–1910.
- Bass, C., Carvalho, R. A., Oliphant, L., Puinean, A. M., Field, L. M., Nauen, R., Williamson, M. S., Moores, G., Gorman, K., 2011. Overexpression of a

- cytochrome P450 monooxygenase, CYP6ER1, is associated with resistance to imidacloprid in the brown planthopper, *Nilaparvata lugens*. *Insect Molecular Biology*. 20(6): 763–773.
- Baughman, R. H., Zakhidov, A. A., Heer, W. A. de., 2002. Carbon Nanotubes-The Route Toward Applications. *Science*. 297(5582): 787–792.
- Bautista, M. A. M., Tanaka, T., Miyata, T., 2007. Identification of permethrin-inducible cytochrome P450s from the diamondback moth, *Plutella xylostella* (L.) and the possibility of involvement in permethrin resistance. *Pesticide Biochemistry and Physiology*. 87(1): 85–93.
- Bautista, M. A., Miyata, T., Miura, K., Tanaka, T., 2009. RNA interference-mediated knockdown of a cytochrome P450, CYP6BG1, from the diamondback moth, *Plutella xylostella*, reduces larval resistance to permethrin. *Insect Biochemistry and Molecular Biology*. 39(1): 38–46.
- Berenbaum, M. R., 2002. Postgenomic chemical ecology: from genetic code to ecological interactions. *Journal of Chemical Ecology*. 28(5):873–896.
- Berenbaum, M. R., Favret, C., Schuler, M. A., 1996. On defining “key innovations” in an adaptive radiation: Cytochrome P450s and *papilionidae*. *The American Naturalist*. 148: S139–S155.
- Bernhardt, R., 2006. Cytochromes P450 as versatile biocatalysts. *Journal of Biotechnology*. 124(1): 128–145.
- Bianco, A., Kostarelos, K., Partidos, C. D., Prato, M., 2005. Biomedical applications of functionalized carbon nanotubes. *Chemical communications (Cambridge, England)*. 5: 571–577.
- Bianco, A., Kostarelos, K., Prato, M., 2005. Applications of carbon nanotubes in drug delivery. *Current Opinion in Chemical Biology*. 9(5):674–679.
- Borm, P. J. A., Robbins, D., Houbold, S., Kuhlisch, T., Fissan, H., Donaldson, K., Schins, R., Stone, V., Kreyling, W., Lademann, J., Krutmann, J., Warheit, D., Oberdorster, E., 2006. The potential risks of nanomaterials: A review carried out for ECETOC. *Particle and Fiber Toxicology*. 3:11.
- Boyer, P. D., Shams, H., Baker, S. L., Mofrad, M. R. K., Islam, M. F., Dahl, K. N., 2016. Enhanced intracellular delivery of small molecules and drugs via non-covalent ternary dispersions of single-wall carbon nanotubes. *Journal of Materials Chemistry B*. 4: 1324–1330.
- Bradley, K., Gabriel, J. C. P., Gruner, G., 2003. Flexible Nanotube Electronics. *Nano Letters*. 3(10): 1353–1355.

- Braydich-Stolle, L. K., Lucas, B., Schrand, A., Murdock, R. C., Lee, T., Schlager, J. J., Hussain, S. M., Hofmann, M. C., 2010. Silver nanoparticles disrupt GDNF/Fyn kinase signaling in spermatogonial stem cells. *Toxicological Sciences*. 116(2): 577–589.
- Buzea, C., Pacheco, I. I., Robbie, K., 2007. Nanomaterials and nanoparticles: Sources and toxicity. *Biointerphases*. 2(4): MR17 - MR71.
- Carino, F. A., Koener, J. P., Plapp Jr., F. W., Feyereisen, R., 1992. Expression of the cytochrome P450 gene CYP6A1 in the housefly, *Musca domestica*. In: Mullin, C. A., Scott, J. G. (Eds.), *Molecular Mechanisms of Insecticide Resistance: Diversity Among Insects*. ACS Symposium Series, 5, Washington, DC, pp. 31–40. Chapter 3.
- Carino, F. A., Koener, J. P., Plapp Jr., F. W., Feyereisen, R., 1994. Constitutive overexpression of the cytochrome P450 gene CYP6A1 in a house fly strain with metabolic resistance to insecticides. *Insect Biochemistry and Molecular Biology*. 2(4): 411–418.
- Castranova, V., Mercer, R. R., 2012. Response to pulmonary exposure to carbon nanotubes. In: Donaldson, K., Duffin, R., Bonner, J., Poland, C., (Eds.), *The Nanotoxicology of Carbon Nanotubes*. Cambridge University Press, Cambridge, pp.134-149. Chapter 8.
- Chen, D., Xi, T., Bai, J., 2007. Biological effects induced by nanosilver particles: *in vivo* study. *Biomedical Materials*. (Bristol, U. K.). 2(3): S126–S128.
- Chen, X., Schluesener, H. J., 2008. Nanosilver: a nanoparticle in medical application. *Toxicology Letters*. 176(1):1-12.
- Cheng, J., Flahaut, E., Cheng, S. H., 2007. Effect of carbon nanotubes on developing zebrafish (*Danio rerio*) embryos. *Environmental Toxicology and Chemistry*. 26(4): 708–716.
- Choi, J. E., Kim, S., Ahn, J. H., Youn, P., Kang, J. S., Park, K., Yi, J., Ryu, D. Y., 2010. Induction of oxidative stress and apoptosis by silver nanoparticles in the liver of adult zebrafish. *Aquatic Toxicology*. 100(2); 151–159.
- Choi, S. J., Oh, J. M., Choy, J. H., 2009. Toxicological effects of inorganic nanoparticles on human lung cancer A549 cells. *Journal of Inorganic Biochemistry*. 103 (3): 463–471.
- Christian, P. 2009. Nanomaterials: Properties, Preparation and Applications. In: Lead, J. R., Smith, E. (Eds.), *Environmental and Human Health Impacts of Nanotechnology*. Blackwell Publishing, John Wiley & Sons, Ltd, Chichester, UK. pp. 31-77. Chapter 2.

- Cohen, M. B., Schuler, M. A., Berenbaum, M. R., 1992. A host-inducible cytochrome P450 from a host-specific caterpillar: molecular cloning and evolution. *Proceedings of the National Academy of Sciences of USA*. 89(22): 10920–10924.
- Colvin, V. L., 2003. The potential environmental impact of engineered nanomaterials. *Nature Biotechnology*. 21(10): 1166–1170.
- Croteau, M. N., Misra, S. K., Luoma, S. N., Jones, E. V., 2011. Silver bioaccumulation dynamics in a freshwater invertebrate after aqueous and dietary exposures to nanosized and ionic Ag. *Environmental Science & Technology*. 45(15): 6600–6607.
- Cui, D., Tian, F., Ozkan, C. S., Wang, M., Gao, H., 2005. Effect of single wall carbon nanotubes on human HEK293 cells. *Toxicology Letters*. 155(1): 73–85.
- Cui, L., Rui, C., Yang, D., Wang, Z., Yuan, H. 2017. De novo transcriptome and expression profile analyses of the Asian corn borer (*Ostrinia furnacalis*) reveals relevant flubendiamide response genes. *BMC Genomics*. 18: 20.
- Daborn, P., Boundy, S., Yen, J., Pittendrigh, B., ffrench-Constant, R., 2001. DDT resistance in *Drosophila* correlates with Cyp6g1 over-expression and confers cross-resistance to the neonicotinoid imidacloprid. *Molecular Genetics and Genomics*. 266(4): 556–563.
- Demir, E., Vales, G., Kaya, B., Creus, A., Marcos, R., 2011. Genotoxic analysis of silver nanoparticles in *Drosophila*. *Nanotoxicology*. 5(3): 417–424.
- Dillon, A. C., Jones, K. M., Bekkedahl, T. A., Kiang, C. H., Bethune, D. S., Heben, M. J., 1997. Storage of hydrogen in single-walled carbon nanotubes. *Nature*. 386, 377–379.
- Ding, Z., Wen, Y., Yang, B., Zhang, Y., Liu, S., Liu, Z., Han, Z., 2013. Biochemical mechanisms of imidacloprid resistance in *Nilaparvata lugens*: over-expression of cytochrome P450 CYP6AY1. *Insect Biochemistry and Molecular Biology*. 43(11): 1021–1027.
- Donaldson, K., Murphy, F.A., Duffin, R., Poland, C. A., 2010. Asbestos, carbon nanotubes and the pleural mesothelium: a review of the hypothesis regarding the role of lung fibre retention in the parietal pleura, inflammation and mesothelioma. *Particle and Fibre Toxicology*. 7:5.
- Dresselhaus, M. S., Dresselhaus, G., Avouris, Ph. 2001. *Carbon Nanotubes: Synthesis, Structure Properties and Applications*. Springer- Verlag: Berlin, 2001. Berlin, 2001. ISBN 978-3-540-39947-6.
- Du, J., Wang, S., You, H., Zhao, X., 2013. Understanding the toxicity of carbon nanotubes in the environment is crucial to the control of nanomaterials in



- producing and processing and the assessment of health risk for human: a review. *Environmental Toxicology and Pharmacology*. 36 (2): 451–462.
- Edwards-Jones V., 2009. The benefits of silver in hygiene, personal care and healthcare. *Letters in Applied Microbiology*. 49(2): 147-152.
- El-Sayed, R., Bhattacharya, K., Gu, Z., Yang, Z., Weber, J. K., Li, H., Leifer, K., Zhao, Y., Toprak, M. S., Zhou, R., Fadeel, B., 2016. Single-Walled Carbon Nanotubes Inhibit the Cytochrome P450 Enzyme, CYP3A4. *Scientific Reports*. 6: 21316.
- Fabrega, J., Luoma, S. M., Tyler, C. R., Galloway, T. S., Lead, J. R., 2011. Silver nanoparticles: behaviour and effects in the aquatic environment. *Environmental International*. 37(2): 517-531.
- Fako, V. E., Furgeson, D. Y., 2009. Zebrafish as a correlative and predictive model for assessing biomaterial nanotoxicity. *Advanced Drug Delivery Reviews*. 61(6): 478–486.
- Feyereisen, R. 2006. Evolution of insect P450. *Biochemical Society Transactions*. 34(6): 1252–1255.
- Feyereisen, R., 2005. Insect cytochrome P450. In: Gilbert, L. I., Iatrou, K., Gill, S.S., (Eds.), *Comprehensive Molecular Insect Science*. Elsevier, Oxford, UK. Vol. 4. pp. 1–77.
- Feyereisen. R., 2012. Insect CYP genes and P450 enzymes. In: Gilbert, L. I., (Eds.), *Insect Molecular Biology and Biochemistry*. London: Academic. pp. 236–316. Chapter 8.
- Fraser, T.W. K., Reinardy, H. C., Shaw, B. J., Henry, T. B., Handy, R. D., 2011. Dietary toxicity of single-walled carbon nanotubes and fullerenes (C60) in rainbow trout (*Oncorhynchus mykiss*). *Nanotoxicology*. 5(1): 98–108.
- Fröhlich, E., Kueznik, T., Samberger, C., Roblegg, E., Wrighton, C., Pieber, T. R., 2010. Size-dependent effects of nanoparticles on the activity of cytochrome P450 isoenzymes. *Toxicology and Applied Pharmacology*. 242(3): 326–332.
- Fu, W., Xie, W., Zhang, Z., Wang, S., Wu, Q., Liu, Y., Zhou, X., Zhou, X., Zhang, Y., 2013. Exploring valid reference genes for quantitative real-time PCR analysis in *Plutella xylostella* (Lepidoptera: *Plutellidae*). *International Journal of Biological Sciences*. 9(8): 792–802.
- Furlong, M. J., Wright, D. J., Dosdall, L. M., 2013. Diamondback moth ecology and management: problems, progress, and prospects. *Annual Review of Entomology*. 58: 517–541.
- Gao, X., Yang, J., Xu, B., Xie, W., Wang, S., Zhang, Y., Yang, F., Wu, Q., 2016. Identification and Characterization of the Gene CYP340W1 from *Plutella*

- xylostella* and Its Possible Involvement in Resistance to Abamectin. International Journal of Molecular Sciences. 17(3): 274.
- Ghafari, P., St-Denis, C. H., Power, M. E., Jin, X., Tsou, V., Mandal, H. S., Bols, N. C., Tang, X., 2008. Impact of carbon nanotubes on the ingestion and digestion of bacteria by ciliated protozoa. Nature Nanotechnology. 3: 347–351.
- Gracia-Espino, E., Sala, G., Pino, F., Halonen, N., Luomahaara, J., Mäklin, J., Tóth, G., Kordás, K., Jantunen, H., Terrones, M., Helistö, P., Seppä, H., Ajayan, P. M., Vajtai, R., 2010. Electrical transport and field-effect transistors using inkjet-printed SWCNT films having different functional side groups. ACS Nano. 4(6): 3318–3324.
- Guzov, V. M., Unnithan, G. C., Chernogolou, A. A., Feyereisen, R., 1998. CYP12A1, a mitochondrial cytochrome P450 from the housefly. Archives of Biochemistry and Biophysics. 359(2): 231–240.
- Haase, A., Tentschert, J., Jungnickel, H., Graf, P., Mantion, A., Draude, F., Plendl, J., Goetz, M. E., Galla, S., Mašić, A., Thuenemann, A. F., Taubert, A., Arlinghaus, H. F., Luch, A., 2011. Toxicity of silver nanoparticles in human macrophages: uptake, intracellular distribution and cellular responses. Journal of Physics: Conference Series. 304(1): 12030.
- Han, J. W., Jeong, J. K., Gurunathan, S., Choi, Y. J., Das, J., Kwon, D. N., Cho, S. G., Park, C., Seo, H. G., Park, J. K., Kim, J. H., 2016. Male- and female-derived somatic and germ cell-specific toxicity of silver nanoparticles in mouse. Nanotoxicology. 10(3): 361–373.
- Hochella, M. F., Lower, S. K., Maurice, P. A., Penn, R. L., Sahai, N., Sparks, D. L., Twining, B. S., 2008. Nanominerals, mineral nanoparticles, and earth systems. Science. 319(5870): 1631–1635.
- Hoet, P. H. M., Bruske-Hohfeld, I., Salata, O. V., 2004. Nanoparticles—Known and unknown health risks. Journal of Nanobiotechnology. 2:1–15.
- Hoi, K. K., Daborn, P. J., Battlay, P., Robin, C., Batterham, P., O’Hair, R. A. J., Donald, W. A., 2014. Dissecting the insect metabolic machinery using twin ion mass spectrometry: a single P450 enzyme metabolizing the insecticide imidacloprid *in vivo*. Analytical Chemistry. 86(7): 3525–3532.
- Holt, B. D., Short, P. A., Rapet, A. D., Wang, Y. L., Islam, M. F., Dahl, K. N., 2010. Carbon nanotubes reorganize actin structures in cells and *ex vivo*. ACS Nano. 4 (8): 4872–4878.
- Hund-Rinke, K., Simon, M., 2006. Ecotoxic effect of photocatalytic active nanoparticles (TiO<sub>2</sub>) on algae and daphnids. Environmental Science and Pollution Research. 13(4): 225–232.

- Hussain, S. M., Hess, K. L., Gearhart, J. M., Geiss, K. T., Schlager, J. J., 2005. In vitro toxicity of nanoparticles in BRL 3A rat liver cells. *Toxicology in Vitro*. 19(7): 975–983.
- Iga, M., Kataoka, H., 2012. Recent studies on insect hormone metabolic pathways mediated by cytochrome P450 enzymes. *Biological & Pharmaceutical Bulletin*. 35(6): 838–843.
- Iijima, S., 1991. Helical microtubules of graphitic carbon. *Nature*. 354: 56-58.
- Iijima, S., Ichihashi, T., 1993. Single-shell carbon nanotubes of 1-nm diameter. *Nature* 363: 603-605.
- Jhonson, D.R., 1953. *Plutellama clipenniesresistance* to DDT in Java. *Journal of Economic Entomology*. 46: 176.
- Ji, J. H., Jung, J. H., Kim, S. S., Yoon, J. U., Park, J. D., Choi, B. S., Chung, Y. H., Kwon, I. H., Jeong, J., Han, B. S., Shin, J. H., Sung, J. H., Song, K. S., Yu, I. J., 2007. Twenty-eight-day inhalation toxicity study of silver nanoparticles in Sprague-Dawley rats. *Inhalation Toxicology*. 19(10): 857–871.
- Jia, G., Wang, H., Yan, L., Wang, X., Pei, R., Yan, T., Zhao, Y., Guo, X., 2005. Cytotoxicity of carbon nanomaterials: single-wall nanotube, multi-wall nanotube, and fullerene. *Environmental Science & Technology*. 39 (5): 1378–1383.
- Jiang, W., Kim, B. Y. S., Rutka, J. T., Chan, W. C. W., 2008. Nanoparticle-mediated cellular response is size-dependent. *Nature Nanotechnology*. 3: 145 – 150.
- Johnston, H. J., Hutchison, G. R., Christensen, F. M., Peters, S., Hankin, S., Aschberger, K., Stone, V., 2010. A critical review of the biological mechanisms underlying the *in vivo* and *in vitro* toxicity of carbon nanotubes: the contribution of physicochemical characteristics. *Nanotoxicology*. 4(2): 207-246.
- Johnston, H. J., Hutchison, G., Christensen, F. M., Peters, S., Hankin, S., Stone, V., 2010. A review of the *in vivo* and *in vitro* toxicity of silver and gold particulates: particle attributes and biological mechanisms responsible for the observed toxicity. *Critical Reviews in Toxicology*. 40(4): 328–46.
- Joviano-Santos, J. V., Sá, M. A., De Maria, M. L., Almeida, T. C. S., Geraldo, V., Oliveira, S., Ladeira, L. O., Ferreira, A. J., 2014. Evaluation of cardiovascular toxicity of carbon nanotubes functionalized with sodium hyaluronate in oral regenerative medicine. *Brazilian Journal of Medical and Biological Research*. 47(7): 560-566.
- Kagan, V. E., Konduru, N. V., Feng, W., Allen, B. L., Conroy, J., Volkov, Y., Vlasova, I. I., Belikova, N. A., Yanamala, N., Kapralov, A., Tyurina, Y. Y., Shi, J., Kisin, E. R., Murray, A. R., Franks, J., Stolz, D., Gou, P., Klein-Seetharaman, J., Fadeel, B., Star, A., Shvedova, A. A., 2010. Carbon nanotubes degraded by neutrophil

- myeloperoxidase induce less pulmonary inflammation. *Nature Nanotechnology*. 5: 354-359.
- Kam, N. W. S., Liu, Z. A., Dai, H. J., 2006. Carbon nanotubes as intracellular transporters for proteins and DNA: an investigation of the uptake mechanism and pathway. *Angewandte Chemie International Edition*. 45 (4): 577–581.
- Kang, S., Pinault, M., Pfefferle, L. D., Elimelech, M., 2007. Single-walled carbon nanotubes exhibit strong antimicrobial activity. *Langmuir*. 23 (17): 8670–8673.
- Karunker, I., Benting, J., Lueke, B., Ponge, T., Nauen, R., Roditakis, E., Vontas, J., Gorman, K., Denholm, I., Morin, S., 2008. Over-expression of cytochrome P450 CYP6CM1 is associated with high resistance to imidacloprid in the B and Q biotypes of *Bemisia tabaci* (Hemiptera: *Aleyrodidae*). *Insect Biochemistry and Molecular Biology*. 38(6): 634–644.
- Kayiran, S. B., Lamari, F. D., Levesque, D., 2004. Adsorption properties and structural characterization of activated carbons and nanocarbons. *The Journal of Physical Chemistry B*. 108(39): 15211-15215.
- Kennedy, A. J., Gunter, J. C., Chappell, M. A., Goss, J. D., Hull, M. S., Kirgan, R. A., Steevens, J. A., 2009. Influence of nanotube preparation in aquatic bioassays. *Environmental Toxicology and Chemistry*. 28: 1930e1938.
- Kim, J., Kim, S., Lee, S., 2011. Differentiation of the toxicities of silver nanoparticles and silver ions to the Japanese medaka (*Oryzias latipes*) and the cladoceran *Daphnia magna*. *Nanotoxicology*. 5(2): 208-214.
- Kim, S. W., Nam, S. H., An, Y. J., 2012. Interaction of Silver Nanoparticles with Biological Surfaces of *Caenorhabditis elegans*. *Ecotoxicology and Environmental Safety*. 7: 64–70.
- Kim, Y. S., Kim, J. S., Cho, H. S., Rha, D. S., Kim, J. M., Park, J. D., Choi, B. S., Lim, R., Chang, H. K., Chung, Y. H., Kwon, I. H., Jeong, J., Han, B. S., Yu, I. J., 2008. Twenty-eight-day oral toxicity, genotoxicity, and gender-related tissue distribution of silver nanoparticles in Sprague–Dawley rats. *Inhalation Toxicology*. 20(6): 575–583.
- Kim, Y. S., Song, M. Y., Park, J. D., Song, K. S., Ryu, H. R., Chung, Y. H., Chang, H. K., Lee, J. H., Oh, K. H., Kelman, B. J., Hwang, I. K., Yu, I. J., 2010. Subchronic oral toxicity of silver nanoparticles. *Particle and Fibre Toxicology*. 7: 20.
- Kisin, E. R., Murray, A. R., Keane, M. J., Shi, X. C., Schwegler-Berry, D., Gorelik, O., Arepalli, S., Castranova, V., Wallace, W. E., Kagan, V. E., Shvedova, A. A., 2007. Single-walled carbon nanotubes: geno- and cytotoxic effects in lung fibroblast V79 cells. *Journal of Toxicology and Environmental Health A*. 70 (24): 2071–2079.

- Klaine, S. J., Alvarez, P. J. J., Batley, G. E., Fernandes, T. F., Handy, R. D., Lyon, D. Y., Mahendra, S., McLaughlin, M. J., Lead, J. R., 2008. Nanomaterials in the Environment: Behavior, Fate, Bioavailability, and Effects. *Environmental Toxicology and Chemistry*. 27(9): 1825-1851.
- Kostarelos, K. L., Lacerda, G., Pastorin, G., Wei, W., Wieckowski, S., Luangsivilay, J., Godefroy, S., Pantarotto, D., Briand, J. P., Muller, S., Prato, M., Bianco, A., 2007. Cellular uptake of functionalized carbon nanotubes is independent of functional group and cell type. *Nature Nanotechnology*. 2: 108–113.
- Kosuge, H., Sherlock, S. P., Kitagawa, T., Dash, R., Robinson, J. T., Dai, H., McConnell, M. V., 2012. Near infrared imaging and photothermal ablation of vascular inflammation using single-walled carbon nanotubes. *Journal of the American Heart Association*. 1(6): e002568.
- Kruss, S., Hilmer, A. J., Zhang, J., Reuel, N. F., Mu, B., Strano, M. S., 2013. Carbon nanotubes as optical biomedical sensors. *Advanced Drug Delivery Reviews*. 65(15): 1933–1950.
- Kulthong, K., Maniratanachote, R., Kobayashi, Y., Fukami, T., Yokoi, T., 2012. Effects of silver nanoparticles on rat hepatic cytochrome P450 enzyme activity. *Xenobiotica*. 42(9): 854–862.
- Kumari, A., Yadav, S. K., Yadav, S. C., 2010. Biodegradable polymeric nanoparticles based drug delivery systems. *Colloids and Surfaces B: Biointerfaces*. 75 (1): 1-18.
- Lacerda, L., Bianco, A., Prato, M., Kostarelos, K., 2006. Carbon nanotubes as nanomedicines: from toxicology to pharmacology. *Advanced Drug Delivery Reviews*. 58 (14): 1460–1470.
- Lam, C. W., James, J. T., McCluskey, R., Hunter, R. L., 2004. Pulmonary toxicity of single-wall carbon nanotubes in mice 7 and 90 days after intratracheal instillation. *Toxicological Sciences*. 77(1): 126–134
- Lamb, J. G., Hathaway, L. B., Munger, M. A., Raucy, J. L., Franklin, M. R., 2010. Nanosilver particle effects on drug metabolism in vitro. *Drug Metabolism and Disposition*. 38(12): 2246–2251.
- Lara, H. H., Garza-Treviño, E. N., Ixtapan-Turrent, L., Singh, D. K., 2011. Silver nanoparticles are broad-spectrum bactericidal and virucidal compounds. *Journal of Nanobiotechnology*. 9: 30.
- Lee, H. Y., Park, H. K., Lee, Y. M., Kim, K., Park, S. B., 2007a. A practical procedure for producing silver nanocoated fabric and its antibacterial evaluation for biomedical applications. *Chemical Communications (Cambridge)*. 28: 2959–2961.

- Lee, K. J., Nallathamby, P. D., Browning, L. M., Osgood, C. J., Xu, X. H. N., 2007b. In vivo imaging of transport and biocompatibility of single silver nanoparticles in early development of zebrafish embryos. *ACS Nano*. 1(2): 133–143.
- Lee, S. H., Lee, H. Y., Lee, E. J., Min, K. J., Khang, D., Min, K. J., 2015. Effects of carbon nanofiber on physiology of *Drosophila*. *International Journal of Nanomedicine*. 10(1): 3687–3697.
- Leeuw, T. K., Reith, R. M., Simonette, R. A., Harden, M. E., Cherukuri, P., Tsyboulski, D. A., Beckingham, K. M., Weisman, R. B., 2007. Single-Walled Carbon Nanotubes in the Intact Organism: Near-IR Imaging and Biocompatibility Studies in *Drosophila*. *Nano Letters*. 7(9): 2650–2654.
- Legramante, J. M., Valentini, F., Magrini, A., Palleschi, G., Sacco, S., Iavicoli, I., Pallante, M., Moscone, D., Galante, A., Bergamaschi, E., Bergamaschi, A., Pietroiusti, A., 2009. Cardiac autonomic regulation after lung exposure to carbon nanotubes. *Human & Experimental Toxicology*. 28(6-7): 369-375.
- Li, H., Dai, H., Wei, H., 2005. Molecular cloning and nucleotide sequence of CYP6BF1 from the diamondback moth, *Plutella xylostella*. *Journal of Insect Science*. 5: 45.
- Liau, S. Y., Read, D. C., Pugh, W. J., Furr, J. R., Russel, A. D., 1997. Interaction of silver nitrate with readily identifiable groups: relationship to the antibacterial action of silver ions. *Letters in Applied Microbiology*. 25(4): 279–283.
- Lim, D., Roh, J. Y., Eom, H. J., Choi, J. Y., Hyun, J., Choi, J., 2012. Oxidative stress-related PMK-1 P38 MAPK activation as a mechanism for toxicity of silver nanoparticles to reproduction in the nematode *Caenorhabditis elegans*. *Environmental Toxicology and Chemistry*. 31(3): 585–592.
- Lin, J., Zhang, H., Chen, Z., Zheng, Y., 2010. Penetration of lipid membranes by gold nanoparticles: insights into cellular uptake, cytotoxicity, and their relationship. *ACS Nano*. 4 (9): 5421–5429.
- Lin, Q., Jin, F., Hu, Z., Chen, H., Yin, F., Li, Z., Dong, X., Zhang, D., Ren, S., Feng, X., 2013. Transcriptome analysis of chlorantraniliprole resistance development in the diamondback moth *Plutella xylostella*. *PLoS One*. 8(8): e72314.
- Liu, C., Fan, Y. Y., Liu, M., Cong, H. T., Cheng, H. T., Dresselhaus, M. S., 1999. Hydrogen storage in single-walled carbon nanotubes at room temperature. *Science*. 2(86): 1127-1129.
- Liu, N., Scott, J. G., 1998. Increased transcription of CYP6D1 causes cytochrome P450-mediated insecticide resistance in housefly. *Insect Biochemistry and Molecular Biology*. 2(8): 531–535.

- Liu, X., Lee, P. Y., Ho, C. M., Lui, V. C., Chen, Y., Che, C. M., Tam, P. K., Wong, K. K., 2010. Silver nanoparticles mediate differential responses in keratinocytes and fibroblasts during skin wound healing. *Chem Med Chem*. 5 (3): 468-75.
- Liu, X., Vinson, D., Abt, D., Hurt, R. H., Rand, D. M., 2009. Differential Toxicity of Carbon Nanomaterials in *Drosophila*: Larval Dietary Uptake Is Benign, but Adult Exposure Causes Locomotor Impairment and Mortality. *Environmental Science & Technology*. 43(16): 6357-6363.
- Long, R. Q., Yang, R. T. 2001b. Carbon nanotubes as a superior sorbent for nitrogen oxides. *Industrial & Engineering Chemistry Research*. 40(20): 4288-4291.
- Long, R. Q., Yang, R. T., 2001a. Carbon nanotubes as superior sorbent for dioxin removal. *Journal of the American Chemical Society*. 12(9): 2058-2059.
- Lowry, G. V., Gregory, K. B., Apte, S. C., Lead, J. R., 2012. Transformations of Nanomaterials in the Environment. *Environmental Science and Technology*. 46(13): 6893-6899.
- Maklin, J., Mustonen, T., Kordas, K., Saukko, S., Toth, G., Vahakangas, J., 2007. Nitric Oxide Gas Sensors with Functionalized Carbon Nanotubes. *Physica Status Solidi (b)*. 244(11): 4298–4302.
- Manna, S. K., Sarkar, S., Barr, J., Wise, K., Barrera, E. V., Jejelowo, O., Rice-Ficht, A. C., Ramesh, G. T., 2005. Single-walled carbon nanotube induces oxidative stress and activates nuclear transcription factor  $\kappa$ B in human keratinocytes. *Nano Letter*. 5(9):1676–1684.
- Martignoni, M., Groothuis, G. M., de Kanter, R., 2006. Species differences between mouse, rat, dog, monkey and human CYP-mediated drug metabolism, inhibition and induction. *Expert Opinion on Drug Metabolism & Toxicology*. 2(6): 875–894.
- Martinez-Paz, P., Morales, M., Martinez-Guitarte, J. L., Morcillo, G., 2012. Characterization of a cytochrome P450 gene (CYP4G) and modulation under different exposures to xenobiotics (tributyltin, nonylphenol, bisphenol A) in *Chironomus riparius* aquatic larvae. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*. 155(2): 333–343.
- Matambo, T. S., Paine, M. J., Coetzee, M., Koekemoer, L. L., 2010. Sequence characterization of cytochrome P450 CYP6P9 in pyrethroid resistant and susceptible *Anopheles funestus* (Diptera: *Culicidae*). *Genetics and Molecular Research*. 9(1): 554–564.
- Mathias, F. T., Romano, R. M., Kizys, M. M., Kasamatsu, T., Giannocco, G., Chiamolera, M. I., Dias-da-Silva, M. R., Romano, M. A., 2015. Daily exposure to silver nanoparticles during prepubertal development decreases adult sperm and reproductive parameters. *Nanotoxicology*. 9(1): 64-70.

- Maynard, A. D., Warheit, D. B., Philbert, M. A., 2011. The new toxicology of sophisticated materials: nanotoxicology and beyond. *Toxicological Science*. 120: S109–S129.
- Meitl, M. A., Zhou, Y. X., Gaur, A., Jeon, S., Usrey, M. L., Strano, M. S., Rogers, J. A., 2004. Solution Casting and Transfer Printing Single-Walled Carbon Nanotube Films. *Nano Letters*. 4: 1643–1647.
- Meyer, J. N., Lord, C. A., Yang, X. Y., Turner, E. A., Badireddy, A. R., Marinakos, S. M., Chilkoti, A., Wiesner, M. R., Auffan, M., 2010. Intracellular uptake and associated toxicity of silver nanoparticles in *Caenorhabditis elegans*. *Aquatic Toxicology*. 100: 140–150.
- Miresmaeili, S. M., Halvaei, I., Fesahat, F., Fallah, A., Nikonahad, N., Taherinejad, M., 2013. Evaluating the role of silver nanoparticles on acrosomal reaction and spermatogenic cells in rat. *Iranian Journal of Reproductive Medicine*. 11: 423–430.
- Moore, M. N., 2006. Do nanoparticles present ecotoxicological risks for the health of the aquatic environment? *Environment International*. 32(9): 967–976.
- Morimoto, Y., Hirohashi, M., Kobayashi, N., Ogami, A., Horie, M., Oyabu, T., Myojo, T., Hashiba, M., Mizuguchi, Y., Kambara, T., Lee, B.W., Kuroda, E., Shimada, M., Wang, W.N., Mizuno, K., Yamamoto, K., Fujita, K., Nakanishi, J., Tanaka, I., 2012. Pulmonary toxicity of well-dispersed single-wall carbon nanotubes after inhalation. *Nanotoxicology*. 6: 766–775.
- Mwangi, J. N., Wang, N., Ingersoll, C. G., Hardesty, D. K., Brunson, E. L., Li, H., Deng, B., 2012. Toxicity of carbon nanotubes to freshwater aquatic invertebrates. *Environmental Toxicology and Chemistry*. 31: 1823–1830.
- Nel, A., Xia, T., Mädler, L., Li, N., 2006. Toxic potential of materials at the nanolevel. *Science*. 311(5761): 622–627.
- Nelson, D. R., 1998. Metazoan cytochrome P450 evolution. *Comparative Biochemistry and Physiology Part: C Pharmacology, Toxicology & Endocrinology*. 121(1-3): 15–22.
- Niu, G., Rupasinghe, S. G., Zangerl, A. R., Siegel, J. P., Schuler, M. A., Berenbaum, M. R., 2011. A substrate-specific cytochrome P450 monooxygenase, CYP6AB11, from the polyphagous navel orangeworm (*Amyelois transitella*). *Insect Biochemistry and Molecular Biology*. 41(4): 244–253.
- Oberdorster, E., 2004. Manufactured nanomaterials (fullerenes, C60) induce oxidative stress in the brain of juvenile largemouth bass. *Environmental Health Perspectives*. 112(10):1058–1062.



- Oberdorster, G., Oberdorster, E., Oberdorster, J., 2005. Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. *Environmental Health Perspectives*. 113(7): 823-839.
- Oberdörster, G., Oberdörster, E., Oberdörster, J., 2007. Concepts of nanoparticle dose metric and response metric. *Environmental Health Perspectives*. 115(6): A290.
- Ong, C., Lee, Q. Y., Cai, Y., Liu, X., Ding, J., Yung, L. Y. L., Bay, B. H., Baeg, G. H., 2016a. Silver nanoparticles disrupt germline stem cell maintenance in the *Drosophila* testis. *Science Reports*. 6: 20632.
- Ong, L. C., Chung, F. F., Tan, Y. F., Leong, C. O., 2016b. Toxicity of single-walled carbon nanotubes. *Archives of Toxicology*. 90(1): 103-118.
- Palanisamy, N. K., Ferina, N., Amirulhusni, A. N., Mohd-Zain, Z., Hussaini, J., Ping, L. J., Durairaj, R., 2014. Antibiofilm properties of chemically synthesized silver nanoparticles found against *Pseudomonas aeruginosa*. *Journal of Nanobiotechnology*. 12:2.
- Park, E. J., Yi, J., Kim, Y., Choi, K., Park, K., 2010. Silver nanoparticles induce cytotoxicity by a Trojan-horse type mechanism. *Toxicology in Vitro*. 24: 872–878.
- Park, K. H., Chhowalla, M., Iqbal, Z., Sesti, F., 2003. Single-walled carbon nanotubes are a new class of ion channel blockers. *Journal of Biological Chemistry*. 278 (50): 50212–50216.
- Pelkonen, O., Mäenpää, J., Taavitsainen, P., Rautio, A., Raunio, H., 1998. Inhibition and induction of human cytochrome P450 (CYP) enzymes. *Xenobiotica*. 28:1203–1253.
- Petersen, E. J., Henry, T. B., 2011. Methodological considerations for testing the ecotoxicity of carbon nanotubes and fullerenes: review. *Environmental Toxicology and Chemistry*. 31: 1– 13.
- Petersen, E. J., Huang, Q., Weber, W. J., 2008. Ecological uptake and depuration of carbon nanotubes by *Lumbriculus variegatus*. *Environmental Health Perspectives*. 116: 496–500.
- Pfaffl, M.W., Horgan, G.W., Dempfle, L., 2002. Relative expression software tool (REST (c)) for group-wise comparison and statistical analysis of relative expression results in real-time PCR. *Nucleic Acids Research*. 30(9): e36.
- Philbrook, N. A., Walker, V. K., Afrooz, A. R. M. N., Saleh, N. B., Winn, L. M., 2011. Investigating the effects of functionalized carbon nanotubes on reproduction and development in *Drosophila melanogaster* and CD-1 mice. *Reproductive Toxicology*. 32: 442–448.

- Posgai, R., Cipolla-McCulloch, C. B., Murphy, K. R., Hussain, S. M., Rowe, J. J., Nielsen, M. G., 2011. Differential toxicity of silver and titanium dioxide nanoparticles on *Drosophila melanogaster* development, reproductive effort, and viability: Size, coatings and antioxidants matter. *Chemosphere*. 85: 34–42.
- Powell, M. C., Kanarek, M. S., 2006. Nanomaterial health effects— Part 1: Background and current knowledge. *Wisconsin Medical Journal*. 105:16– 20.
- Poynton, H. C., Lazorchak, J. M., Impellitteri, C. A., Blalock, B. J., Rogers, K., Allen, H. J., Loguinov, A., Heckman, J. L., Govindasmaw, S., 2012. Toxicogenomic responses of nanotoxicity in *Daphnia magna* exposed to silver nitrate and coated silver nanoparticles. *Environmental Science & Technology*. 46: 6288–6296.
- Puinean, A. M., Foster, S. P., Oliphant, L., Denholm, I., Field, L. M., Millar, N. S., Williamson, M. S., Bass, C., 2010. Amplification of a cytochrome P450 gene is associated with resistance to neonicotinoid insecticides in the aphid *Myzus persicae*. *PloS Genet*. 6(6): e1000999.
- Rahman, M. F., Wang, J., Patterson, T. A., Saini, U. T., Robinson, B. L., Newport, G. D., Murdock, R. C., Schlager, J. J., Hussain, S. M., Ali, S. F., 2009. Expression of genes related to oxidative stress in the mouse brain after exposure to silver-25 nanoparticles. *Toxicology Letters*. 187: 15–21.
- Ratte, H.T., 1999. Bioaccumulation and toxicity of silver compounds: a review. *Environmental Toxicology and Chemistry*. 18: 89–108.
- Risom, L., Moller, P., Loft, S., 2005. Oxidative stress-induced DNA damage by particulate air pollution. *Mutation Research*. 592: 119-137.
- Roberts, A. P., Mount, A. S., Seda, B., Souther, J., Qiao, R., Lin, S., Pu, C. K., Rao, A. M., Klaine, S. J., 2007. In vivo biomodification of lipid-coated carbon nanotubes by *Daphnia magna*. *Environmental Science & Technology*. 41: 3028–3029.
- Robinson, J. T., Hong, G., Liang, Y., Zhang, B., Yaghi, O. K., Dai, H., 2012. In vivo fluorescence imaging in the second near-infrared window with long circulating carbon nanotubes capable of ultrahigh tumor uptake. *Journal of the American Chemical Society*. 134: 10664–10669.
- Rodrigues, D. F., Elimelech, M., 2010. Toxic effects of single-walled carbon nanotubes in the development of *E. coli* biofilm. *Environmental Science & Technology*. 44 (12), 4583–4589.
- Rogers, N. J., Franklin, N. M., Apte, S. C., Batley, G. E. 2007. The importance of physical and chemical characterization in nanoparticle toxicity studies. *Integrated Environmental Assessment and Management*. 3(2): 303-4

- Rueckes, T., Kim, K., Joselivich, E., Tseng, G. Y., Cheung, C. L., Lieber, C.M., 2000. Carbon nanotubes based random access memory for molecular computing. *Science*. 289: 94-97.
- Sahu, S. C., Casciano, D. A., 2009. *Nanotoxicology from In Vivo and In Vitro Models to Health Risks*. John Wiley & Sons, New York, pp. 427–457.
- Sargent, L. M., Shvedova, A. A., Hubbs, A. F., Salisbury, J. L., Benkovic, S. A., Kashon, M. L., Lowry, D. T., Murray, A. R., Kisin, E. R., Friend, S., McKinstry, K. T., Battelli, L., Reynolds, S. H., 2009. Induction of aneuploidy by single-walled carbon nanotubes. *Environmental and Molecular Mutagenesis*. 5 (8): 708–717.
- Sayes, C. M., Liang, F., Hudson, J. L., Mendez, J., Guo, W., Beach, J. M., Moore, V. C., Doyle, C. D., West, J. L., Billups, W. E., Ausman, K. D., Colvin, V. L., 2006. Functionalization density dependence of single-walled carbon nanotubes cytotoxicity in vitro. *Toxicology Letters*. 161:135–142.
- Scientific Committee on Emerging and Newly Identified Health Risks. 2007. The appropriateness of the risk assessment methodology in accordance with the Technical Guidance Documents for new and existing substances for assessing the risks of nanomaterials, 21–22 June 2007. European Commission, Brussels, Belgium.
- Scott, J. G., 1999. Cytochromes P450 and insecticide resistance. *Insect Biochemistry and Molecular Biology*. 29: 757–777.
- Scott, J. G., Wen, Z., 2001. Cytochromes P450 of insects: the tip of the iceberg. *Pest Management Science*. 57: 958–967.
- Scown, T. M., Santos, E. M., Johnston, B. D., Gaiser, B., Baalousha, M., Mitov, S., Lead, J. R., Stone, V., Fernandes, T. F., Jepson, M., van Aerle, R., Tyler, C. R., 2010. Effects of aqueous exposure to silver nanoparticles of different sizes in rainbow trout. *Toxicological Sciences*. 115: 521–534.
- Sereemasapun, A., Hongpiticharoen, P., Rojanathanes, R., Maneewattanapinyo, P., Ekgasit, S., Warisnoicharoen, W., 2008. Inhibition of human cytochrome P450 enzymes by metallic nanoparticles: a preliminary to nanogenomics. *International Journal of Pharmacology*. 4: 492–495.
- Shahverdi, A. R., Fakhimi, A., Shahverdi, H. R., Minaian, S., 2007. Synthesis and effect of silver nanoparticles on the antibacterial activity of different antibiotics against *Staphylococcus aureus* and *Escherichia coli*. *Nanomedicine*. 3:168.
- Sharifi, S., Behzadi, S., Laurent, S., Forrest, M. L., Stroeve, P., Mahmoud, M., 2012. Toxicity of nanomaterials. *Chemical Society Reviews*. 41: 2323–2343.

- Shen, B., Zhao, D., Qiao, C., Lan, W., 2004. Cloning of CYP9G2 from the Diamondback Moth, *Plutella xylostella* (Lepidoptera: Yponomeutidae). *DNA Seq.* 15(3): 228–233.
- Shi, J. P., Evans, D. E., Khan, A. A., Harrison, R. M., 2001. Sources and concentrations of nanoparticles (<10 nm diameter) in the urban atmosphere. *Atmospheric Environment.* 35: 1193–1202.
- Shvedova, A. A., Kisin, E. R., Mercer, R., Murray, A. R., Johnson, V. J., Gorelik, O., Arepalli, S., Hubbs, A. F., Mercer, R. R., Keohavong, P., Sussman, N., Jin, J. J., Yin, J., Stone, B., Chen, B. T., Deye, G., Maynard, A., Castranova, V., Baron, P., Kagan, V. E., 2008. Inhalation vs. aspiration of single-walled carbon nanotubes in C57BL/6 mice: inflammation, fibrosis, oxidative stress, and mutagenesis. *American Journal of Physiology Lung Cellular and Molecular Physiology.* 295(4): L552-L565.
- Shvedova, A. A., Kisin, E. R., Murray, A. R., Johnson, V. J., Potapovich, A. I., Tyurina, Y. Y., Gorelik, O., Arepalli, S., Schwegler-Berry, D., Hubbs, A. F., Antonini, J., Evans, D. E., Ku, B. K., Ramsey, D., Maynard, A., Kagan, V. E., Castranova, V., Baron, P., 2005. Unusual inflammatory and fibrogenic pulmonary responses to single-walled carbon nanotubes in mice. *American Journal of Physiology Lung Cellular and Molecular Physiology.* 289: L698-L708.
- Shvedova, A. A., Yanamala, N., Kisin, E. R., Tkach A. V., Murray, A. R., Hubbs, A., Chirila, M. M., Keohavong, P., Sycheva, L. P., Kagan, V. A., Castranova, V., 2014. Long-term effects of carbon containing nanomaterials and asbestos in the lung: one year postexposure comparisons. *American Journal of Physiology Lung Cellular and Molecular Physiology.* 306: L170-182.
- Silver Key, S. C., Reaves, D., Turner, F., Bang, J. J., 2011. Impacts of Silver Nanoparticle Ingestion on Pigmentation and Developmental Progression in *Drosophila*. *Atlas Journal of Biology.* 1: 52–61.
- Smith, C. J., Shaw, B. J., Handy, R. D., 2007. Toxicity of single walled carbon nanotubes to rainbow trout, (*Oncorhynchus mykiss*): Respiratory toxicity, organ pathologies, and other physiological effects. *Aquatic Toxicology.* 82: 94–109.
- Snow, E. S., Novak, J. P., Campbell, P. M., Park, D., 2003. Random Networks of Carbon Nanotubes as an Electronic Material. *Applied Physics Letters.* 82: 2145–2147.
- Sohn, E. K., Chung, Y. S., Johari, S. A., Kim, T. G., Kim, J. K., Lee, J. H., Lee, Y. H., Kang, S. W., Yu, I. J., 2015. Acute toxicity comparison of single-walled carbon nanotubes in various freshwater organisms. *Biomed Research International.* 2015: 323090.
- Stevenson, B. J., Bibby, J., Pignatelli, P., Muangnoicharoen, S., O'Neill, P.M., Lian, L. Y., Müller, P., Nikou, D., Steven, A., Hemingway, J., Sutcliffe, M. J., Paine, M. J., 2011. Cytochrome P450 6M2 from the malaria vector *Anopheles gambiae*

- metabolizes pyrethroids: Sequential metabolism of deltamethrin revealed. *Insect Biochemistry and Molecular Biology*. 41(7): 492–502.
- Sun, C. N., 1992. Insecticide resistance in diamondback moth, in “Diamondback Moth and Other Crucifer Pests: Proceedings of the Second International Workshop” (N. S. Talekar, Ed.), pp. 419–426, Asian Vegetable Research and Development Center, Taiwan.
- Sun, C. N., Wu, T. K., Chen, J. S., Lee, W. T., 1986. Insecticide resistance in diamondback moth, in “Diamondback Moth Management: Proceedings of the First International Workshop” (N. S. Talekar, Ed.), pp. 359–371, Asian Vegetable Research and Development Center, Taiwan.
- Sun, Y. G., Xia, Y., 2003. Gold and silver nanoparticles: A class of chromophores with colors tunable in the range from 400 to 750 nm. *Analyst* (Cambridge, U.K.). 128(6): 686–691.
- Sun, Y. P., Fu, K. F., Lin, Y., Huang, W. J., 2002. Functionalized carbon nanotubes: Properties and applications. *Accounts of Chemical Research*. 35: 1096–1104.
- Tabashnik, B. E., Liu, Y. B., Finson, N., Masson, L., Heckel, D. G., 1997. One gene in diamondback moth confers resistance to four *Bacillus thuringiensis* toxins. *Proceedings of the National Academy of Sciences of the United States of America*. 94(5): 1640–1644.
- Talekar, N. S., Shelton, A. M., 1993. Biology, ecology, and management of the diamondback Moth. *Annual Review of Entomology*. 38: 275–301.
- Talekar, N. S., Yang, J. C., Lee, S. T., 1990. Compilers of Annotated Bibliography of Diamondback Moth. Volume 2. Asian Vegetable Research and Development Center, Shanhua, Taiwan: pp. 199.
- Tang, L. J., Xiong, S., Wang, J., Wang, L., Liu, J., Li, F., Yuan, T. Xi., 2009. Distribution, translocation and accumulation of silver nanoparticles in rats. *Journal of Nanoscience and Nanotechnology*. 9: 4924–4932.
- Teeguarden, J. G., Webb-Robertson, B. J., Waters, K. M., Murray, A. R., Kisin, E. R., Varnum, S., Jakobs, J. M., Pounds, J. G., Zanger, R. C., Shvedova, A. A., 2011. Comparative proteomics and pulmonary toxicity of instilled single-walled carbon nanotubes, crocidolite asbestos, and ultrafine carbon black in mice. *Toxicological Science*. 120: 123–135.
- Templeton, R. C., Ferguson, P. L., Washburn, K. M., Scrivens, W. A., Chandler, G. T., 2006. Life-cycle effects of single-walled carbon nanotubes (SWNTs) on an estuarine meiobenthic copepod. *Environmental Science & Technology*. 40: 7387–7393.

- Tian, F., Cui, D., Schwarz, H., Estrada, G.G., Kobayashi, H., 2006. Cytotoxicity of single-wall carbon nanotubes on human fibroblasts. *Toxicology in Vitro*. 20 (7): 1202–1212.
- Tomita, T., Liu, N., Smith, F. F., Sridhar, P., Scott, J. G., 1995. Molecular mechanisms involved in increased expression of a cytochrome P450 responsible for pyrethroid resistance in the housefly, *Musca domestica*. *Insect Molecular Biology*. 4: 135–140.
- Tong, H., McGee, J. K., Saxena, R. K., Kodavanti, U. P., Devlin, R. B., Gilmour, M. I., 1420 2009. Influence of acid functionalization on the cardiopulmonary toxicity of carbon nanotubes and carbon black particles in mice. *Toxicology and Applied Pharmacology*. 239: 224-232.
- Vance, M. E., Kuiken, T., Vejerano, E. P., McGinnis, S. P., Hochella, M. F., Hull, D. R., Rejeski, D., Hull, M. S., Hull, M. S., 2015. Nanotechnology in the real world: Redeveloping the nanomaterial consumer products inventory. *Beilstein Journal of Nanotechnology*. 6: 1769–1780.
- Wagner, V., Dullaart, A., Bock, A. K., Zweck, A., 2006. The emerging nanomedicine landscape. *Nature Biotechnology*. 24(10): 1211-1217.
- Warisnoicharoen, W., Hongpiticharoen, P., Lawanprasert, S., 2011. Alteration in enzymatic function of human cytochrome P450 by silver nanoparticles. *Research Journal of Environmental Toxicology*. 5: 58–64.
- Werck-Reichhart, D., Feyereisen, R., 2000. Cytochromes P450: a success story. *Genome Biology*. 1(6): Reviews 3003.
- Winder, A. J., Harris, H., 1991. New assays for the tyrosine hydroxylase and dopa oxidase activities of tyrosinase. *European Journal of Biochemistry*. 198: 317–26.
- Yamanaka, M., Hara, K., Kudo, J., 2005. Bactericidal actions of a silver ion solution on *Escherichia coli*, studied by energy-filtering transmission electron microscopy and proteomic analysis. *Applied and Environmental Microbiology*. 71(11):7589-7593.
- Yang, L., Watts, D. J., 2005. Particle surface characteristics may play an important role in phytotoxicity of alumina nanoparticles. *Toxicology Letters*. 158:122–132.
- Yee, W., Selvaduray, G., Hawkins, B., 2015. Characterization of silver nanoparticle-infused tissue adhesive for ophthalmic use. *Journal of the Mechanical Behavior of Biomedical Materials*. 55: 67–74.
- Yoon, K.Y., Byeon, J. H., Park, J. H., Hwang, J., 2007. Susceptibility constants of *Escherichia coli* and *Bacillus subtilis* to silver and copper nanoparticles. *Science of the Total Environment*. 373: 572–575.

- Yu, L., Tang, W., He, W., Ma, X., Vasseur, L., Baxter, S.W., Yang, G., Huang, S., Song, F., You, M., 2015. Characterization and expression of the cytochrome P450 gene family in diamondback moth, *Plutella xylostella* (L.). *Scientific Reports*. 5: 8952.
- Zhao, C. M., Wang, W. X., 2011. Comparison of acute and chronic toxicity of silver nanoparticles and silver nitrate to *Daphnia magna*. *Environmental Toxicology and Chemistry*. 30: 885–892.
- Zhou, X. J., Qian, K., Tong, Y., Zhu, J. J., Qiu, X. H., Zeng, X. P. 2014. De novo transcriptome of the hemimetabolous German cockroach (*Blattella germanica*). *PLoS ONE*. 9:e106932.
- Zhou, Y. X., Gaur, A., Hur, S. H., Kocabas, C., Meitl, M. A., Shim, M., Rogers, J. A., 2004. p-Channel, n-Channel Thin Film Transistors and p-n Diodes Based on Single Wall Carbon Nanotube Networks. *Nano Letters*. 4: 2031–2035.
- Zhu, F., Moural, T. W., Nelson, D. R., Palli, S. R., 2016. A specialist herbivore pest adaptation to xenobiotics through up-regulation of multiple Cytochrome P450s. *Scientific Reports*. 6:20421.
- Zhu, F., Moural, T. W., Shah, K., Palli, S. R., 2013. Integrated analysis of cytochrome P450 gene superfamily in the red flour beetle, *Tribolium castaneum*. *BMC Genomics*. 14: 174.
- Zhu, F., Parthasarathy, R., Bai, H., Woithe, K., Kaussmann, M., Nauen, R., Harrison, D. A., Palli, S. R., 2010. A brain-specific cytochrome P450 responsible for the majority of deltamethrin resistance in the QTC279 strain of *Tribolium castaneum*. *Proceedings of the National Academy of Sciences of the United States of America*. 107: 8557–8562.
- Zhu, X., Zhu, L., Chen, Y., Tian, S., 2009. Acute toxicities of six manufactured nanomaterial suspensions to *Daphnia magna*. *Journal of Nanoparticle Research*. 11: 67–75.
- Zimmer, C. T., Bass, C., Williamson, M. S., Kaussmann, M., Wölfel, K., Gutbrod, O., Nauen, R., 2014. Molecular and functional characterization of CYP6BQ23, a cytochrome P450 conferring resistance to pyrethroids in European populations of pollen beetle, *Meligethes aeneus*. *Insect Biochemistry and Molecular Biology*. 45: 18–29.
- Zuo, G., Huang, Q., Wie, G., Zhou, R., Fang, H., 2010. Plugging into proteins: poisoning protein function by a hydrophobic nanoparticle. *ACS Nano*. 4 (12): 7508–7514.

Table 1: The concentrations of single walled carbon nanotube (SWCNT) and AgNPs tested and the number of replicates for each feeding trial.

ENP	Trial	Treatments	No. of replicates
SWCNT	1	0 µg/ml, 8.64 µg/ml, 17.28 µg/ml, NLC, NFC	4x
	2	0 µg/ml, 8.64 µg/ml, 17.28 µg/ml, NLC, NFC	4x
	3	0 µg/ml, 17.28 µg/ml, 34.56 µg/ml, NLC, NFC	8x
	4	0 µg/ml, 17.28 µg/ml, 34.56 µg/ml, 69.12 µg/ml, NLC, NFC	5x
	5	0 µg/ml, 34.56 µg/ml, 69.12 µg/ml, 138.24 µg/ml, NLC, NFC	5x
	6	0 µg/ml, 138.24 µg/ml, NLC, NFC	5x
AgNP	1	0 µg/ml, 4.32 µg/ml, 8.64 µg/ml, NLC, NFC	5x
	2	0 µg/ml, 4.32 µg/ml, 8.64 µg/ml, NLC, NFC	5x

Table 2: Primer design and properties used for qRT-PCR

CYP6BG1		Amplicon length	T <sub>m</sub>	GC content
Forward	ACCCTCGAGAAGGGTCTCCGA	111	61.7	61.9
Reverse	ATTCTCCGGCGAAAACCGATC		57.7	52.4
RPL32				
Forward	CAATTTACCGCCCTACCATC	91	53.4	50
Reverse	CGCCAGTTACGCTTTATTTTG		52.7	42.9



Table 3: Mean ( $\pm$ SE) consumption by diamondback moths (DMB) of feed without and with increasing concentrations of SWCNTs across six trials. Different letters (a- c) represent significant differences (\* for  $p \leq 0.05$ , \*\* for  $P \leq 0.01$  and \*\*\* for  $P \leq 0.001$ ) among different SWCNT concentration (0  $\mu\text{g/ml}$ , 8.64  $\mu\text{g/ml}$ , 17.28  $\mu\text{g/ml}$ , 34.56  $\mu\text{g/ml}$ , 69.12  $\mu\text{g/ml}$ , and 138.24  $\mu\text{g/ml}$ ).

Trial	Concentration of SWCNT in artificial feed					
	0 $\mu\text{g/ml}$	8.64 $\mu\text{g/ml}$	17.28 $\mu\text{g/ml}$	34.56 $\mu\text{g/ml}$	69.12 $\mu\text{g/ml}$	138.24 $\mu\text{g/ml}$
1	1432.45 $\pm 53.0$	1408.23 $\pm 40.3$	1466.73 $\pm 75.1$			
2	1577.01 $\pm 40.1$	1596.24 $\pm 11.1$	1564.01 $\pm 15.5$			
3*	1718.83 $\pm 25.4^a$		1847.16 $\pm 41.5^b$	1802.92 $\pm 12.2^{a,b}$		
4***	1408.42 $\pm 13.9^a$		1336.4 $\pm 12.2^b$	1307.74 $\pm 16.8^b$	1407.75 $\pm 9.3^a$	
5***	1214.90 $\pm 12.6^a$			1430.51 $\pm 44.9^{b,c}$	1530.31 $\pm 63.9^c$	1296.69 $\pm 19.9^{a,b}$
6	1462.22 $\pm 69.7$					1486.83 $\pm 137$

Table 4: Mean ( $\pm$ SE) growth of diamondback moths (DMB) feeding on artificial feed without and with increasing concentrations of SWCNTs. Different letters (a- b) represent significant differences (\* for  $p \leq 0.05$ ) among different SWCNT concentration (0  $\mu\text{g/ml}$ , 8.64  $\mu\text{g/ml}$ , 17.28  $\mu\text{g/ml}$ , 34.56  $\mu\text{g/ml}$ , 69.12  $\mu\text{g/ml}$ , and 138.24  $\mu\text{g/ml}$ ).

Trial	Concentration of SWCNT in artificial feed					
	0 $\mu\text{g/ml}$	8.64 $\mu\text{g/ml}$	17.28 $\mu\text{g/ml}$	34.56 $\mu\text{g/ml}$	69.12 $\mu\text{g/ml}$	138.24 $\mu\text{g/ml}$
1*	96.94 $\pm 3.6^a$	77.05 $\pm 6.3^b$	79.79 $\pm 2.3^{a,b}$			
2	94.08 $\pm 4.9$	95.90 $\pm 5.9$	93.30 $\pm 6.1$			
3	95.30 $\pm 4.9$		100.74 $\pm 4.7$	95.92 $\pm 4.2$		
4	107.60 $\pm 3.1$		110.88 $\pm 1.8$	104.88 $\pm 3.0$	110.48 $\pm 2.8$	
5	108.77 $\pm 3.9$			104.84 $\pm 4.5$	93.65 $\pm 6.5$	99.07 $\pm 3.2$
6*	110.67 $\pm 3.0^a$					99.90 $\pm 2.0^b$

Table 5: Mean ( $\pm$ SE) % of pupation of diamondback moth (DBM) across six different trials for different SWCNT concentration (0  $\mu$ g/ml, 8.64  $\mu$ g/ml, 17.28  $\mu$ g/ml, 34.56  $\mu$ g/ml, 69.12  $\mu$ g/ml, and 138.24  $\mu$ g/ml).

Trial	Concentration of SWCNT in artificial feed					
	0 $\mu$ g/ml	8.64 $\mu$ g/ml	17.28 $\mu$ g/ml	34.56 $\mu$ g/ml	69.12 $\mu$ g/ml	138.24 $\mu$ g/ml
1	96.67 $\pm$ 1.92	100.00 $\pm$ 0.00	95.00 $\pm$ 1.67			
2	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00			
3	99.17 $\pm$ 0.83		95.00 $\pm$ 2.44	96.67 $\pm$ 2.52		
4	100.00 $\pm$ 0.00		94.67 $\pm$ 3.89	97.33 $\pm$ 1.63	96.00 $\pm$ 1.63	
5	100.00 $\pm$ 0.00			96.00 $\pm$ 1.63	92.00 $\pm$ 4.90	96.00 $\pm$ 1.63
6	100.00 $\pm$ 0.00					96.00 $\pm$ 2.45

Table 6: Mean ( $\pm$ SE) % of survival of DBM across six different trials for different SWCNT concentration (0  $\mu$ g/ml, 8.64  $\mu$ g/ml, 17.28  $\mu$ g/ml, 34.56  $\mu$ g/ml, 69.12  $\mu$ g/ml, and 138.24  $\mu$ g/ml).

Trial	Concentration of SWCNT in artificial feed					
	0 $\mu$ g/ml	8.64 $\mu$ g/ml	17.28 $\mu$ g/ml	34.56 $\mu$ g/ml	69.12 $\mu$ g/ml	138.24 $\mu$ g/ml
1	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00			
2	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00			
3	100.00 $\pm$ 0.00		100.00 $\pm$ 0.00	100.00 $\pm$ 0.00		
4	89.42 $\pm$ 3.42		86.38 $\pm$ 4.82	86.38 $\pm$ 3.61	92.86 $\pm$ 3.91	
5	100.00 $\pm$ 0.00			97.24 $\pm$ 1.96	90.94 $\pm$ 4.31	98.57 $\pm$ 1.43
6	96.00 $\pm$ 2.45					91.78 $\pm$ 2.07

Table 7: Percentage of  $\mu\text{g}$  growth ( $\pm\text{SE}$ ) per  $\mu\text{g}$  consumption for Diamondback Moth across six different trials. Different letters (a- c) represent significant differences (\* for  $p \leq 0.05$ , \*\* for  $P \leq 0.01$  and \*\*\* for  $P \leq 0.001$ ) among different SWCNT concentration (0  $\mu\text{g/ml}$ , 8.64  $\mu\text{g/ml}$ , 17.28  $\mu\text{g/ml}$ , 34.56  $\mu\text{g/ml}$ , 69.12  $\mu\text{g/ml}$ , and 138.24  $\mu\text{g/ml}$ ).

Trial	Concentration of SWCNT in artificial feed					
	0 $\mu\text{g/ml}$	8.64 $\mu\text{g/ml}$	17.28 $\mu\text{g/ml}$	34.56 $\mu\text{g/ml}$	69.12 $\mu\text{g/ml}$	138.24 $\mu\text{g/ml}$
1 *	6.77 $\pm 0.1^a$	5.47 $\pm 0.4^b$	5.47 $\pm 0.2^b$			
2	5.99 $\pm 0.4$	6.01 $\pm 0.4$	5.97 $\pm 0.4$			
3	5.54 $\pm 0.3$		5.47 $\pm 0.3$	5.3 3 $\pm 0.2$		
4	7.64 $\pm 0.2$		8.30 $\pm 0.1$	8.02 $\pm 0.2$	7.85 $\pm 0.2$	
5 ***	8.95 $\pm 0.3^a$			7.38 $\pm 0.5^{b, c}$	6.12 $\pm 0.3^c$	7.65 $\pm 0.3^{a, b}$
6	7.64 $\pm 0.4$					6.92 $\pm 0.6$

Table 8: Number of eggs per adult ( $\pm\text{SE}$ ) for Diamondback Moth across three different trials. Different letters (a-b) represent significant differences (\* for  $p \leq 0.05$ ) among different SWCNT concentration (0  $\mu\text{g/ml}$ , 8.64  $\mu\text{g/ml}$ , 17.28  $\mu\text{g/ml}$ , 34.56  $\mu\text{g/ml}$ , 69.12  $\mu\text{g/ml}$ , and 138.24  $\mu\text{g/ml}$ ).

Trial	Concentration of SWCNT in artificial feed					
	0 $\mu\text{g/ml}$	8.64 $\mu\text{g/ml}$	7.28 $\mu\text{g/ml}$	34.56 $\mu\text{g/ml}$	69.12 $\mu\text{g/ml}$	138.24 $\mu\text{g/ml}$
4	11.9 $\pm 4.0$		5.7 $\pm 2.4$	13.0 $\pm 2.8$	15.1 $\pm 6.5$	
5 *	27.4 $\pm 4.4^a$			24.9 $\pm 3.9^{a, b}$	17.6 $\pm 5.6^{a, b}$	9.2 $\pm 2.9^b$
6	19.7 $\pm 5.7$					10.9 $\pm 3.6$

Table 9: Mean ( $\pm$ SE) data for different variables of Diamondback Moth across six different trials. Different letters (a- c) represent significant differences (\* for  $p \leq 0.05$ , \*\* for  $P \leq 0.01$  and \*\*\* for  $P \leq 0.001$ ) among different SWCNT concentration (0  $\mu\text{g/ml}$ , 8.64  $\mu\text{g/ml}$ , 17.28  $\mu\text{g/ml}$ , 34.56  $\mu\text{g/ml}$ , 69.12  $\mu\text{g/ml}$ , and 138.24  $\mu\text{g/ml}$ ). In case of egg/adult and conversion, its statistically significant, but Tukey t-test at 95% confidence interval did not show any grouping (Appendices 7 and 14).

Variables	Concentration of SWCNT in artificial feed					
	0 $\mu\text{g/ml}$	8.64 $\mu\text{g/ml}$	17.28 $\mu\text{g/ml}$	34.56 $\mu\text{g/ml}$	69.12 $\mu\text{g/ml}$	138.24 $\mu\text{g/ml}$
Consumption	1490.84 $\pm 33.9$	1502.23 $\pm 40.5$	1599.16 $\pm 50.8$	1561.92 $\pm 55.1$	1469.03 $\pm 36.7$	1391.76 $\pm 72.6$
Growth*	101.99 $\pm 2.1^a$	86.47 $\pm 5.3^b$	97.74 $\pm 3.1^{a, b}$	100.89 $\pm 2.5^{a, b}$	102.06 $\pm 4.4^{a, b}$	99.48 $\pm 1.8^{a, b}$
% Pupation**	99.36 $\pm 0.4^a$	100 $\pm 0.0^{a, b}$	95.87 $\pm 1.3^{a, b}$	96.67 $\pm 1.2^{a, b}$	94.00 $\pm 2.5^b$	96.00 $\pm 1.4^{a, b}$
% survival	97.65 $\pm 9.4$	100 $\pm 0.0$	96.76 $\pm 1.7$	95.45 $\pm 1.7$	91.90 $\pm 2.8$	95.17 $\pm 1.6$
Egg/ Adult*	19.66 $\pm 3.0$		5.7 $\pm 2.4$	18.94 $\pm 3.0$	16.36 $\pm 4.1$	10.07 $\pm 2.2$
Conversion*	6.99 $\pm 0.2$	5.74 $\pm 0.3$	6.24 $\pm 0.3$	6.49 $\pm 0.3$	6.98 $\pm 0.3$	7.29 $\pm 0.3$

Table 10: Mean ( $\pm$ SE) consumption rate of AgNPs on DBM across two different trials. Different letters (a- c) represent significant differences (\* for  $p \leq 0.05$ , \*\* for  $P \leq 0.01$  and \*\*\* for  $P \leq 0.001$ ) among different AgNPs concentration (0  $\mu\text{g/ml}$ , 4.32  $\mu\text{g/ml}$  and 8.64  $\mu\text{g/ml}$ ).

Trial	Concentration of AgNP in artificial feed		
	0 $\mu\text{g/ml}$	4.32 $\mu\text{g/ml}$	8.64 $\mu\text{g/ml}$
1*	1462.22 $\pm$ 69.7 <sup>a</sup>	1309.96 $\pm$ 61.3 <sup>a, b</sup>	1182.54 $\pm$ 27.3 <sup>b</sup>
2***	1617.5 $\pm$ 24.9 <sup>a</sup>	1317.6 $\pm$ 20.6 <sup>c</sup>	1456.0 $\pm$ 22.8 <sup>b</sup>

Table 11: Mean ( $\pm$ SE) growth rate of Diamondback Moth across two different trials among different AgNPs concentration (0  $\mu$ g/ml, 4.32  $\mu$ g/ml and 8.64  $\mu$ g/ml).

Trial	Concentration of AgNP in artificial feed		
	0 $\mu$ g/ml	4.32 $\mu$ g/ml	8.64 $\mu$ g/ml
1	110.67 $\pm$ 2.95	92.90 $\pm$ 9.61	87.12 $\pm$ 8.13
2	81.41 $\pm$ 5.83	68.62 $\pm$ 5.60	75.44 $\pm$ 5.75

Table 12: Mean ( $\pm$ SE) pupation (%) rate of Diamondback Moth across two different trials. Different letters (a- b) represent significant differences (\*\* for  $p \leq 0.01$ ) among different AgNPs concentration (0  $\mu$ g/ml, 4.32  $\mu$ g/ml and 8.64  $\mu$ g/ml).

Trial	Concentration of AgNP in artificial feed		
	0 $\mu$ g/ml	4.32 $\mu$ g/ml	8.64 $\mu$ g/ml
1**	100.00 $\pm$ 0.00 <sup>a</sup>	100.00 $\pm$ 0.00 <sup>a</sup>	90.00 $\pm$ 3.16 <sup>b</sup>
2	100 $\pm$ 0.00	98.00 $\pm$ 2.00	98.00 $\pm$ 2.00

Table 13: Mean ( $\pm$ SE) survival (%) rate of Diamondback Moth across two different trials. Different letters (a- b) represent significant differences (\*\* for  $p \leq 0.01$ ) among different AgNPs concentration (0  $\mu$ g/ml, 4.32  $\mu$ g/ml and 8.64  $\mu$ g/ml).

Trial	Concentration of AgNP in artificial feed		
	0 $\mu$ g/ml	4.32 $\mu$ g/ml	8.64 $\mu$ g/ml
1	96.00 $\pm$ 2.45	100.00 $\pm$ 0.00	95.28 $\pm$ 2.90
2	100 $\pm$ 0.00	94.00 $\pm$ 4.00	100.00 $\pm$ 0.00

Table 14: Percentage of  $\mu\text{g}$  growth per  $\mu\text{g}$  consumption for Diamondback Moth across two different trials among different AgNPs concentration (0  $\mu\text{g/ml}$ , 4.32  $\mu\text{g/ml}$  and 8.64  $\mu\text{g/ml}$ ).

Trial	Concentration of AgNP in artificial feed		
	0 $\mu\text{g/ml}$	4.32 $\mu\text{g/ml}$	8.64 $\mu\text{g/ml}$
1	7.64 $\pm$ 0.42	7.10 $\pm$ 0.71	7.32 $\pm$ 0.54
2	5.02 $\pm$ 0.31	5.23 $\pm$ 0.48	5.18 $\pm$ 0.38

Table 15: Number of eggs per adult for DBM across three different trials among different AgNPs concentration (0  $\mu\text{g/ml}$ , 4.32  $\mu\text{g/ml}$  and 8.64  $\mu\text{g/ml}$ ).

Trial	Concentration of AgNP in artificial feed		
	0 $\mu\text{g/ml}$	4.32 $\mu\text{g/ml}$	8.64 $\mu\text{g/ml}$
1	19.74 $\pm$ 5.69	40.20 $\pm$ 10.1	33.42 $\pm$ 10.4
2	29.52 $\pm$ 7.65	28.91 $\pm$ 3.70	29.32 $\pm$ 5.00



Table 16: Mean ( $\pm$ SE) data for different variables of Diamondback Moth across two different trials. Different letters (a- b) represent significant differences (\* for  $p \leq 0.05$  and \*\* for  $P \leq 0.01$ ) among different AgNPs concentration (0  $\mu\text{g/ml}$ , 4.32  $\mu\text{g/ml}$  and 8.64  $\mu\text{g/ml}$ ).

Variables	Concentration of AgNP in artificial feed		
	0 $\mu\text{g/ml}$	4.32 $\mu\text{g/ml}$	8.64 $\mu\text{g/ml}$
Consumption **	1539.9 $\pm$ 43.5 <sup>a</sup>	1313.8 $\pm$ 30.5 <sup>b</sup>	1319.3 $\pm$ 48.6 <sup>b</sup>
Growth	96.04 $\pm$ 5.7	80.76 $\pm$ 6.62	81.28 $\pm$ 5.08
%Pupation *	100.00 $\pm$ 0.0 <sup>a</sup>	99.00 $\pm$ 1.0 <sup>a</sup>	94.00 $\pm$ 2.2 <sup>b</sup>
% Survival	98.00 $\pm$ 1.3	97.00 $\pm$ 2.13	97.64 $\pm$ 1.6
Egg/Adult	24.63 $\pm$ 4.8	34.55 $\pm$ 5.4	31.37 $\pm$ 5.5
Conversion	6.33 $\pm$ 0.50	6.16 $\pm$ 0.51	6.25 $\pm$ 0.47



1.a. Control feed



1.c. SWCNT feed



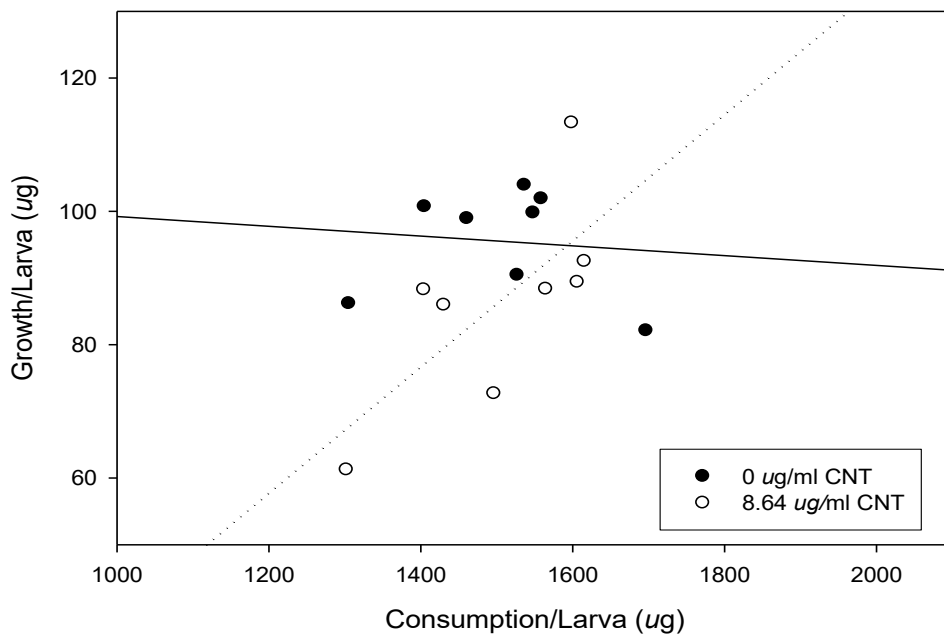
1.b. Control feed larva



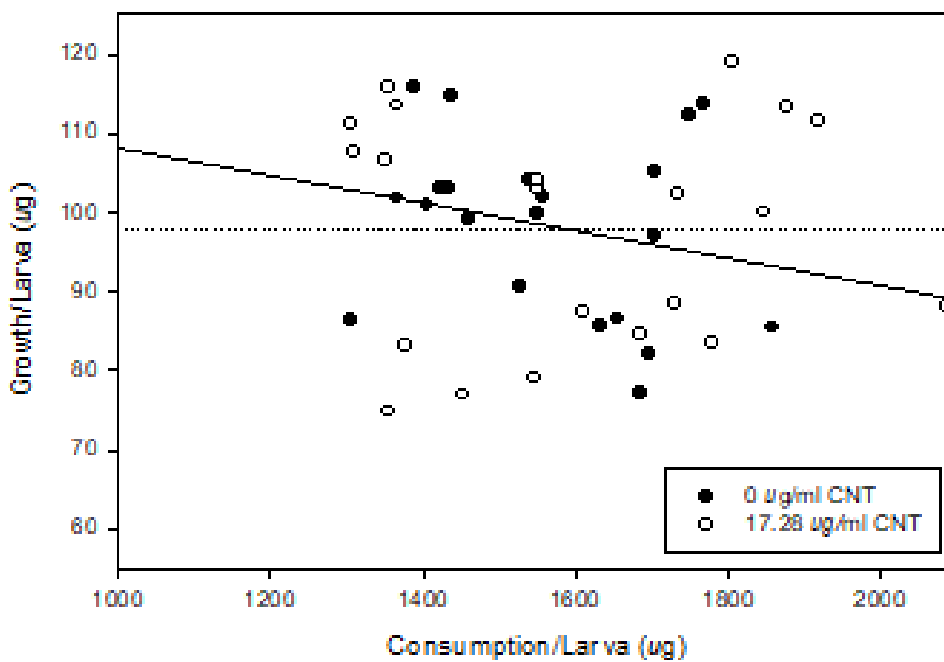
1.d. SWCNT feed larva

Figure 1: DBM larvae exposed to artificial feed. (1.a.) Control feed with larvae. (1.c.) SWCNT feed at higher concentration ( $138.24 \mu\text{g/ml}$ ) with larvae. The SWCNT feed are very much darker than control feed. (1.b.) Larvae feeding on control feed, clear gut. (1.d.) Larvae feed on SWCNT feed, accumulated SWCNT on their gut. Evidence that SWCNT were taken with feed and accumulated in their body.

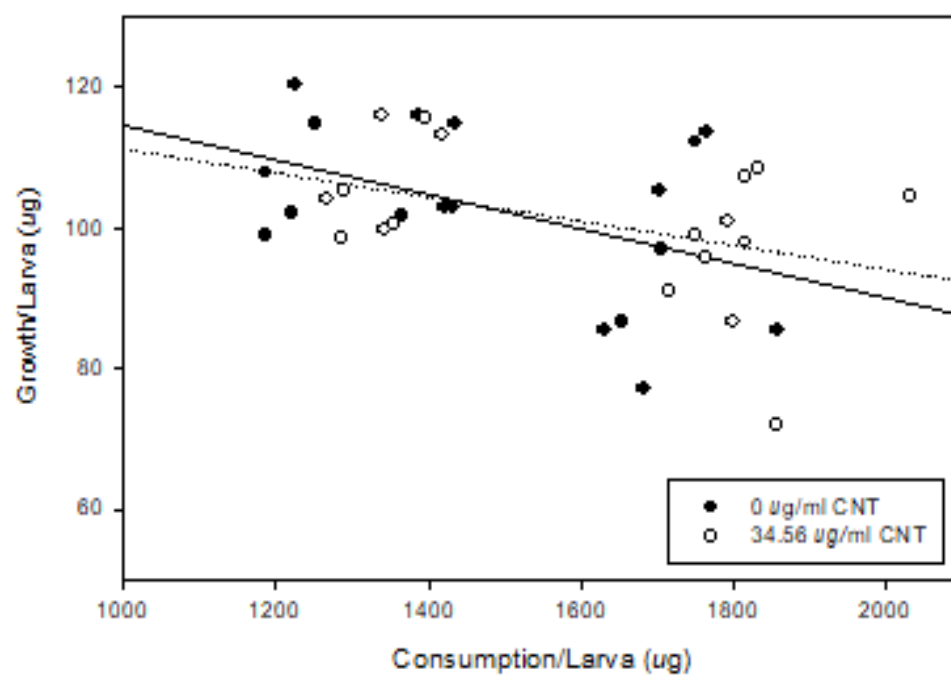
a



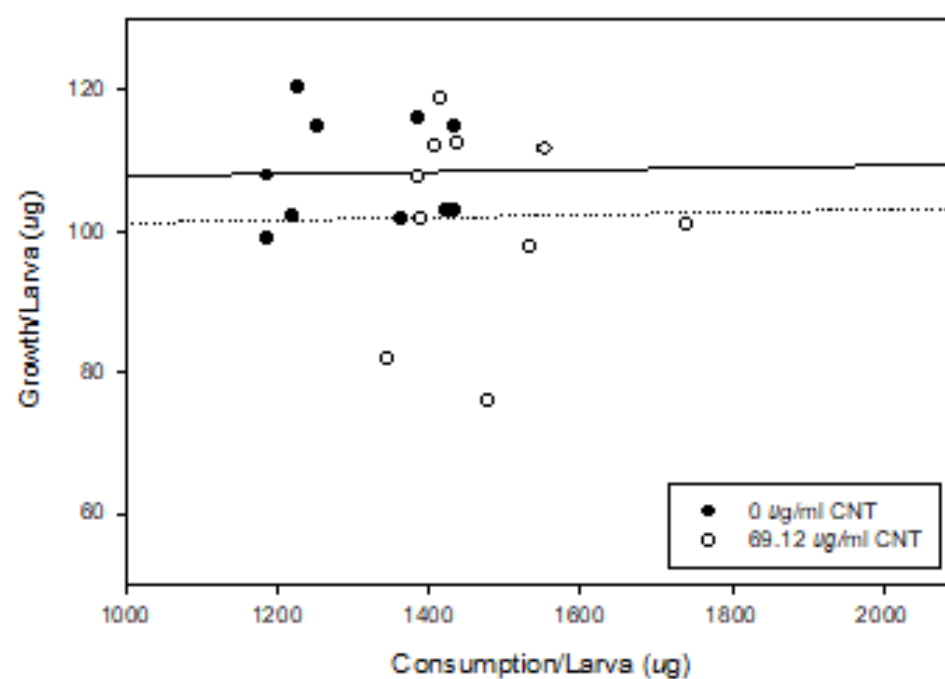
b



c



d



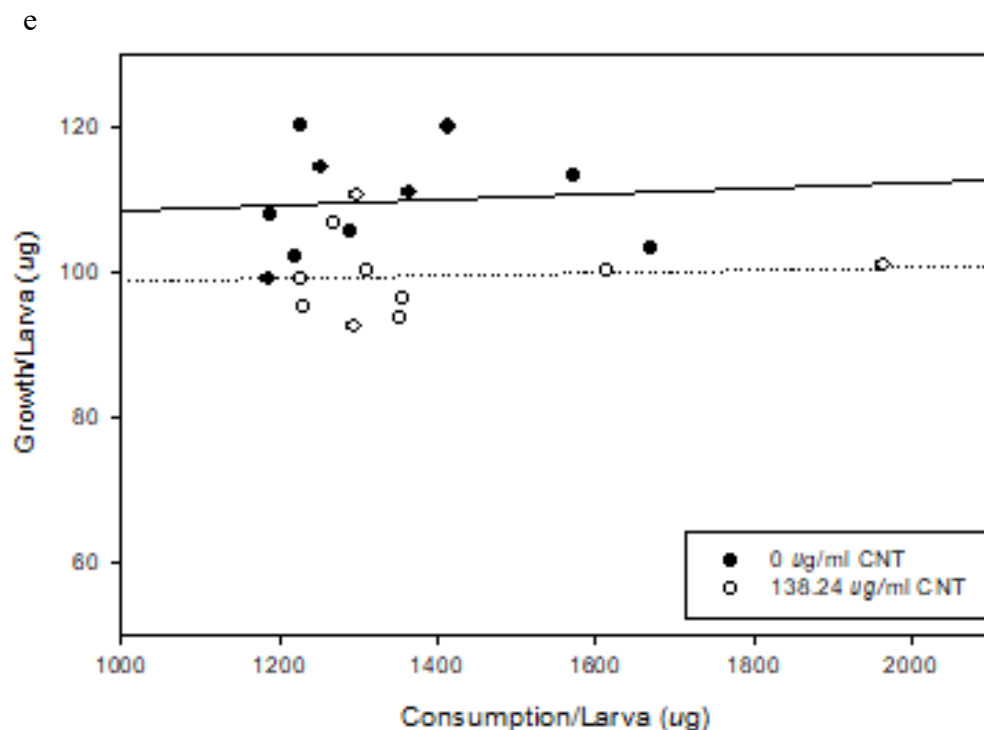


Figure 2. Scatter plot of growth/larva ( $\mu\text{g}$ ) for consumption/larva ( $\mu\text{g}$ ) of diamondback moth exposed to different SWCNT concentration. The average of controls for different SWCNT concentrations are different, as there were several trials for different concentrations. a) 0  $\mu\text{g/ml}$  and 8.64  $\mu\text{g/ml}$ . b) 0  $\mu\text{g/ml}$  and 17.28  $\mu\text{g/ml}$ . c) 0  $\mu\text{g/ml}$  and 34.56  $\mu\text{g/ml}$ . d) 0  $\mu\text{g/ml}$  and 69.12  $\mu\text{g/ml}$ . e) 0  $\mu\text{g/ml}$  and 138.24  $\mu\text{g/ml}$ . Solid line denotes the regression for 0  $\mu\text{g/ml}$  and dotted line denotes the regression for SWCNT concentration.



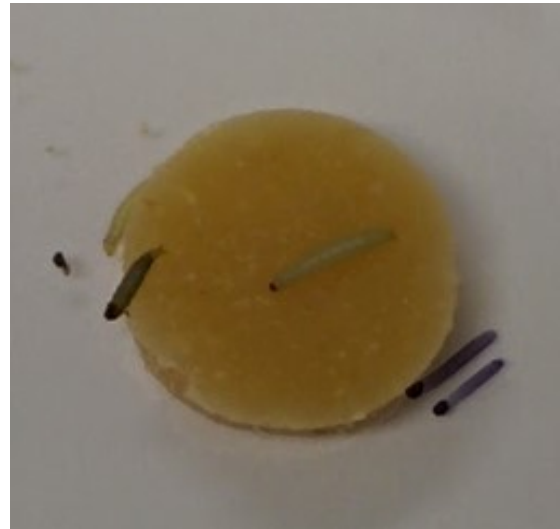
3.a. Control feed



3.c. AgNP feed



3.b. Control feed larva



3.d. AgNP feed larva

Figure 3: DBM larvae exposed to artificial feed. (3.a.) Control feed with larvae. (3.c.) AgNP feed at higher concentration ( $8.64 \mu\text{g/ml}$ ) with larvae. The AgNP feed are a little brownish than control feed. (3.b.) Larvae feeding on control feed, healthy and normal length. (3.d.) Larvae feed on AgNP feed, a dying larva with shorter length.

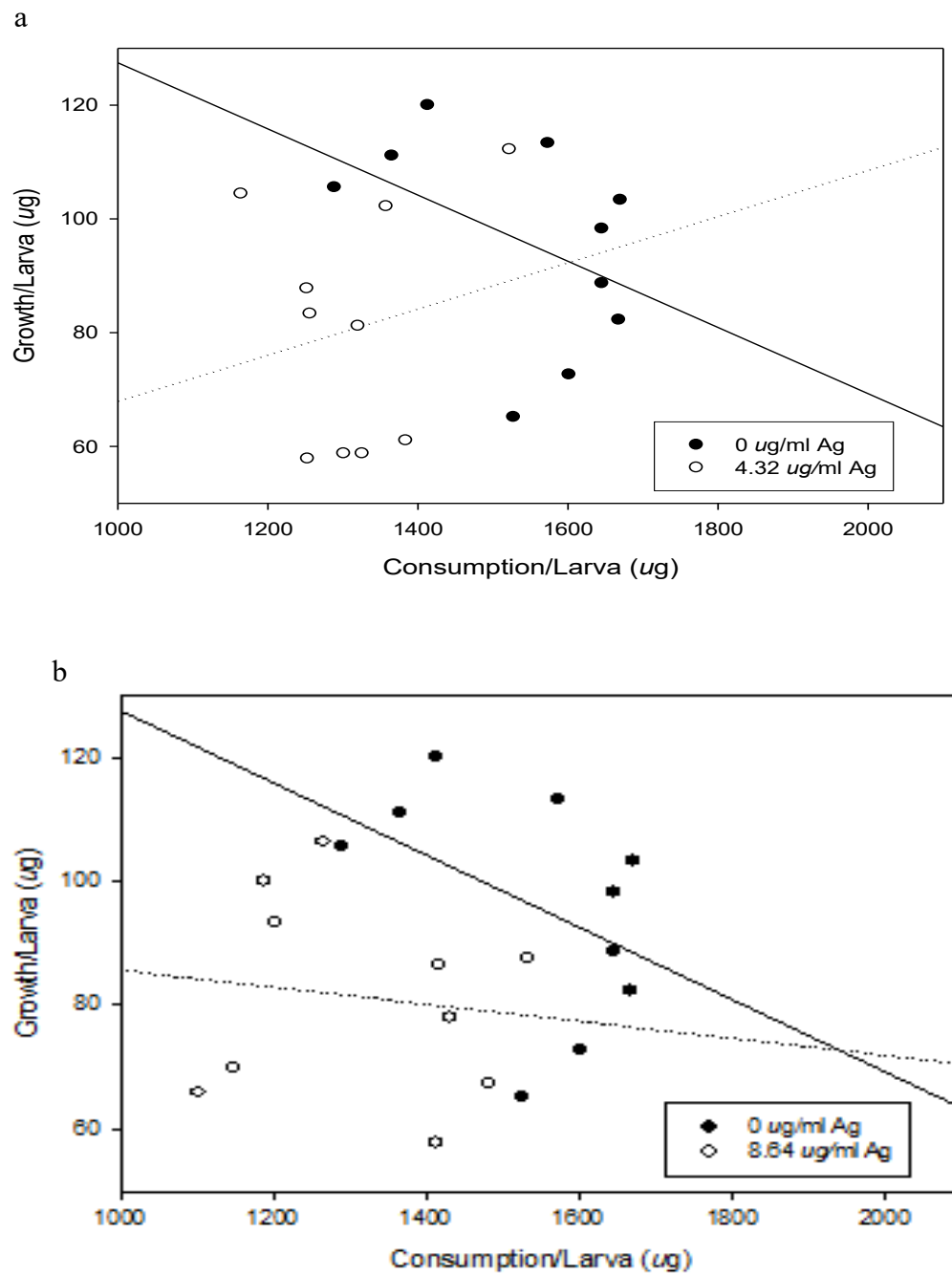


Figure 4. Scatter plot of growth/larva ( $\mu\text{g}$ ) for consumption/larva ( $\mu\text{g}$ ) of diamondback moth exposed to different AgNPs concentration, a) 0  $\mu\text{g/ml}$  and 4.32  $\mu\text{g/ml}$ , b) 0  $\mu\text{g/ml}$  and 8.64  $\mu\text{g/ml}$ . Solid lines denotes the regression for 0  $\mu\text{g/ml}$  and dotted lines denotes the regression for AgNPs.

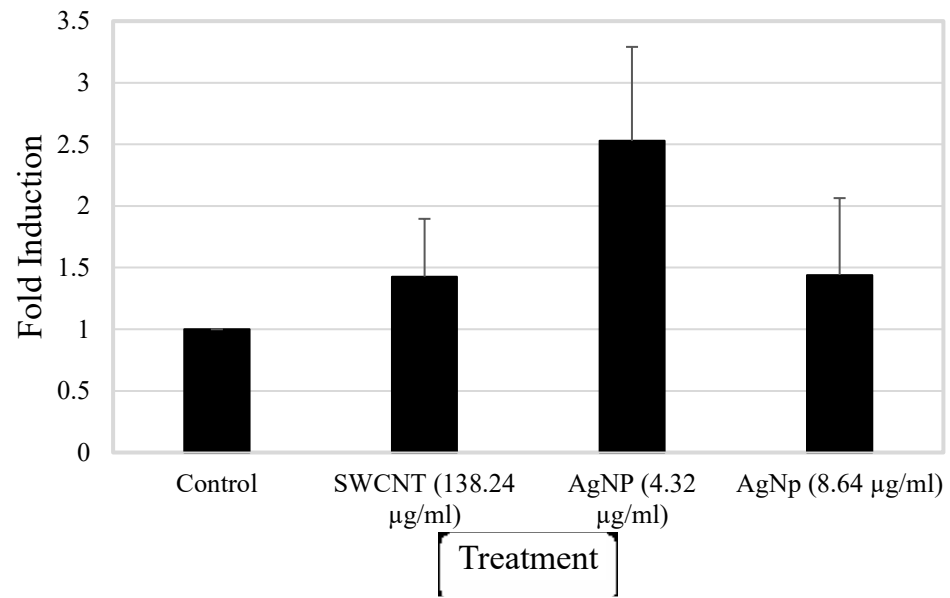


Figure 5: CYP6BG1 gene expression level in DMB feeding on different concentrations and types of ENPs



## APPENDIX

1: Mean ( $\pm$ SE) consumption, growth, pupation, and survival of DBMs feeding on artificial food without or with SWCNTs (8.64  $\mu$ g/ml and 17.28  $\mu$ g/ml) from feeding trial 1. For this trial, consumption (df = 2; F = 0.26 and p = 0.779), pupation (df = 2; F = 03.00 and p = 0.100) and survival was not significantly affected by the presence of SWCNTs in the food. Growth (df = 2; F = 6.00 and p = 0.022) was significantly affected by the presence of SWCNTs in the food. Different letters (a- c) represent significant differences in means within a measured variable and between control and different SWCNT concentrations as determined using Tukey's test.

Variable	Treatment	Mean	SE Mean
Consumption/L/H ( $\mu$ g)	0 $\mu$ g/ml SWCNT	1432.45	53.00
	8.64 $\mu$ g/ml SWCNT	1408.23	40.30
	17.28 $\mu$ g/ml SWCNT	1466.73	75.10
Growth/L/H ( $\mu$ g) *	0 $\mu$ g/ml SWCNT	96.94 <sup>a</sup>	3.60
	8.64 $\mu$ g/ml SWCNT	77.05 <sup>b</sup>	6.30
	17.28 $\mu$ g/ml SWCNT	79.79 <sup>a, b</sup>	2.30
% Pupation	0 $\mu$ g/ml SWCNT	96.67	1.92
	8.64 $\mu$ g/ml SWCNT	100	0
	17.28 $\mu$ g/ml SWCNT	95	1.67
% Survival	0 $\mu$ g/ml SWCNT	100	0
	8.64 $\mu$ g/ml SWCNT	100	0
	17.28 $\mu$ g/ml SWCNT	100	0

2: Mean ( $\pm$ SE) consumption, growth, pupation, and survival of DBM feeding on artificial food without or with SWCNT (8.64  $\mu$ g/ml and 17.28  $\mu$ g/ml) from feeding trial 2. For this trial, consumption (df = 2; F = 0.4 and p = 0.681), growth (df = 2; F = 0.06 and p = 0.946), pupation and survival was not significantly affected by the presence of SWCNTs in the food. Different letters (a- c) represent significant differences in means within a measured variable and between control and different SWCNT concentrations as determined using Tukey's test.

Variable	Treatment	Mean	SE Mean
Consumption/L/H ( $\mu$ g)	0 $\mu$ g/ml SWCNT	1577.01	40.10
	8.64 $\mu$ g/ml SWCNT	1596.24	11.10
	17.28 $\mu$ g/ml SWCNT	1564.01	15.50
Growth/L/H ( $\mu$ g)	0 $\mu$ g/ml SWCNT	94.08	4.90
	8.64 $\mu$ g/ml SWCNT	95.90	5.90
	17.28 $\mu$ g/ml SWCNT	93.30	6.10
% Pupation	0 $\mu$ g/ml SWCNT	100	0
	8.64 $\mu$ g/ml SWCNT	100	0
	17.28 $\mu$ g/ml SWCNT	100	0
% Survival	0 $\mu$ g/ml SWCNT	100	0
	8.64 $\mu$ g/ml SWCNT	100	0
	17.28 $\mu$ g/ml SWCNT	100	0

3: Mean ( $\pm$ SE) consumption, growth, pupation, and survival of DBM feeding on artificial food without or with SWCNTs (17.28  $\mu$ g/ml and 34.56  $\mu$ g/ml) from feeding trial 3. For this trial, growth (df = 2; F = 0.42 and p = 0.661), pupation (df = 2; F = 1.02 and p = 0.379) and survival was not significantly affected by the presence of SWCNTs in the food. Consumption (df = 2; F = 5.08 and p = 0.016) was significantly affected by the presence of SWCNTs in the food. Different letters (a- c) represent significant differences in means within a measured variable and between control and different SWCNT concentrations as determined using Tukey's test.

Variable	Treatment	Mean	SE Mean
Consumption/L/H ( $\mu$ g) *	0 $\mu$ g/ml SWCNT	1718.83 <sup>a</sup>	25.40
	17.28 $\mu$ g/ml SWCNT	1847.16 <sup>b</sup>	41.50
	34.56 $\mu$ g/ml SWCNT	1802.92 <sup>a, b</sup>	12.20
Growth/L/H ( $\mu$ g)	0 $\mu$ g/ml SWCNT	95.30	4.90
	17.28 $\mu$ g/ml SWCNT	100.74	4.70
	34.56 $\mu$ g/ml SWCNT	95.92	4.20
% Pupation	0 $\mu$ g/ml SWCNT	99.17	0.83
	8.64 $\mu$ g/ml SWCNT	95.00	2.44
	17.28 $\mu$ g/ml SWCNT	96.67	2.52
% Survival	0 $\mu$ g/ml SWCNT	100	0
	17.28 $\mu$ g/ml SWCNT	100	0
	34.56 $\mu$ g/ml SWCNT	100	0

4: Mean ( $\pm$ SE) consumption, growth, pupation, survival and egg/adult of DBM feeding on artificial food without or with SWCNTs (17.28  $\mu$ g/ml, 34.56  $\mu$ g/ml, and 69.12  $\mu$ g/ml) from feeding trial 4. For this trial, growth (df = 3; F = 1.06 and p = 0.393), pupation (df = 2; F = 1.01 and p = 0.412), survival (df = 2; F = 0.60 and p = 0.624, and egg/adult (df = 3; F = 0.91 and p = 0.457) was not significantly affected by the presence of SWCNTs in the food. Consumption (df = 3; F = 14.65 and p  $\leq$  0.0001) was highly significantly affected by the presence of SWCNTs in the food. Different letters (a- c) represent significant differences in means within a measured variable and between control and different SWCNT concentrations as determined using Tukey's test.

Variable	Treatment	Mean	SE Mean
Consumption/L/H ( $\mu$ g) ***	0 $\mu$ g/ml SWCNT	1408.42 <sup>a</sup>	13.90
	17.28 $\mu$ g/ml SWCNT	1336.41 <sup>b</sup>	12.20
	34.56 $\mu$ g/ml SWCNT	1307.74 <sup>b</sup>	16.80
	69.12 $\mu$ g/ml SWCNT	1407.75 <sup>a</sup>	9.30
Growth/L/H ( $\mu$ g)	0 $\mu$ g/ml SWCNT	107.60	3.10
	17.28 $\mu$ g/ml SWCNT	110.88	1.80
	34.56 $\mu$ g/ml SWCNT	104.88	3.00
	69.12 $\mu$ g/ml SWCNT	110.48	2.80
% Pupation	0 $\mu$ g/ml SWCNT	100	0
	17.28 $\mu$ g/ml SWCNT	94.67	3.89
	34.56 $\mu$ g/ml SWCNT	97.33	1.63
	69.12 $\mu$ g/ml SWCNT	96.00	1.63
% Survival	0 $\mu$ g/ml SWCNT	89.42	3.42
	17.28 $\mu$ g/ml SWCNT	86.38	4.82
	34.56 $\mu$ g/ml SWCNT	86.38	3.61
	69.12 $\mu$ g/ml SWCNT	92.86	3.91
Egg/Adult	0 $\mu$ g/ml SWCNT	11.9	4.00
	17.28 $\mu$ g/ml SWCNT	5.7	2.40
	34.56 $\mu$ g/ml SWCNT	13.0	2.80
	69.12 $\mu$ g/ml SWCNT	15.1	6.50

5: Mean ( $\pm$ SE) consumption, growth, pupation, survival and egg/adult of DBMS feeding on artificial food without or with SWCNTs (34.56  $\mu$ g/ml, 69.12  $\mu$ g/ml and 138.24  $\mu$ g/ml) from feeding trial 5. For this trial, growth (df = 3; F = 1.99 and p = 0.156), pupation (df = 3; F = 1.45 and p = 0.264) and survival (df = 3; F = 2.73 and p = 0.079) was not significantly affected by the presence of SWCNTs in the food. Consumption (df = 3; F = 11.77 and p  $\leq$  0.0001) and egg/adult (df = 3; F = 3.63 and p = 0.036) was significantly affected by the presence of SWCNTs in the food. Different letters (a- c) represent significant differences in means within a measured variable and between control and different SWCNT concentrations as determined using Tukey's test.

Variable	Treatment	Mean	SE Mean
Consumption/L/H ( $\mu$ g) ***	0 $\mu$ g/ml SWCNT	1214.90 <sup>a</sup>	12.60
	34.56 $\mu$ g/ml SWCNT	1430.51 <sup>b, c</sup>	44.90
	69.12 $\mu$ g/ml SWCNT	1530.31 <sup>c</sup>	63.90
	138.24 $\mu$ g/ml SWCNT	1296.69 <sup>a, b</sup>	19.90
Growth/L/H ( $\mu$ g)	0 $\mu$ g/ml SWCNT	108.77	3.90
	34.56 $\mu$ g/ml SWCNT	104.84	4.50
	69.12 $\mu$ g/ml SWCNT	93.65	6.50
	138.24 $\mu$ g/ml SWCNT	99.07	3.20
% Pupation	0 $\mu$ g/ml SWCNT	100	0
	34.56 $\mu$ g/ml SWCNT	96.0	1.63
	69.12 $\mu$ g/ml SWCNT	92.0	4.90
	138.24 $\mu$ g/ml SWCNT	96.0	1.63
% Survival	0 $\mu$ g/ml SWCNT	100	0
	34.56 $\mu$ g/ml SWCNT	97.24	1.96
	69.12 $\mu$ g/ml SWCNT	90.94	4.31
	138.24 $\mu$ g/ml SWCNT	98.57	1.43
Egg/Adult <sup>*</sup>	0 $\mu$ g/ml SWCNT	27.4 <sup>a</sup>	4.40
	34.56 $\mu$ g/ml SWCNT	24.9 <sup>a, b</sup>	3.90
	69.12 $\mu$ g/ml SWCNT	17.6 <sup>a, b</sup>	5.60
	138.24 $\mu$ g/ml SWCNT	9.2 <sup>b</sup>	2.90

6: Mean ( $\pm$ SE) consumption, growth, pupation, and survival of DBM feeding on artificial food without or with SWCNTs (138.24  $\mu$ g/ml) from feeding trial 6. For this trial, consumption (df = 1; F = 0.3 and p = 0.877), pupation (df = 1; F = 2.67 and p = 0.141), survival (df = 1; F = 1.74 and p = 0.224) and egg/adult (df = 1; F = 1.69 and p = 0.230) was not significantly affected by the presence of SWCNTs in the food. Growth (df = 1; F = 9.1 and p = 0.017) was significantly affected by the presence of SWCNTs in the food. Different letters (a- c) represent significant differences in means within a measured variable and between control and different SWCNT concentrations as determined using Tukey's test.

Variable	Treatment	Mean	SE Mean
Consumption/L/H ( $\mu$ g)	0 $\mu$ g/ml SWCNT	1462.22	69.70
	138.24 $\mu$ g/ml SWCNT	1486.83	137.00
Growth/L/H ( $\mu$ g) *	0 $\mu$ g/ml SWCNT	110.67 <sup>a</sup>	3.00
	138.24 $\mu$ g/ml SWCNT	99.90 <sup>b</sup>	2.00
% Pupation	0 $\mu$ g/ml SWCNT	100.0	0
	138.24 $\mu$ g/ml SWCNT	96.0	2.45
% Survival	0 $\mu$ g/ml SWCNT	96.0	2.45
	138.24 $\mu$ g/ml SWCNT	91.78	2.07
Egg/Adult	0 $\mu$ g/ml SWCNT	19.7	5.70
	138.24 $\mu$ g/ml SWCNT	10.9	3.60

7: Mean ( $\pm$ SE) consumption, growth, pupation, survival and egg/adult of DBM feeding on artificial food without or with SWCNTs (8.64  $\mu$ g/ml, 17.28  $\mu$ g/ml, 34.56  $\mu$ g/ml, 69.12  $\mu$ g/ml and 138.24  $\mu$ g/ml) from all combined feeding trial. For this trial, consumption (df = 5; F = 1.86 and p = 0.109) and survival (df = 5; F = 1.87 and p = 0.108) was not significantly affected by the presence of SWCNTs in the food. Growth (df = 5; F = 2.33 and p = 0.049), pupation (df = 5; F = 3.22 and p = 0.01) and egg/adult were statistically significant (df = 4; F = 2.72 and p = 0.041) was significantly affected by the presence of SWCNTs in the food. Different letters (a- c) represent significant differences in means within a measured variable and between control and different SWCNT concentrations as determined using Tukey's test.

Variable	Treatment	Mean	SE Mean
Consumption/L/H ( $\mu$ g)	0 $\mu$ g/ml SWCNT	1490.84	33.90
	8.64 $\mu$ g/ml SWCNT	1502.23	40.50
	17.28 $\mu$ g/ml SWCNT	1599.16	50.80
	34.56 $\mu$ g/ml SWCNT	1561.92	55.10
	69.12 $\mu$ g/ml SWCNT	1469.03	36.70
	138.24 $\mu$ g/ml SWCNT	1391.76	72.60
Growth/L/H ( $\mu$ g) *	0 $\mu$ g/ml SWCNT	101.99 <sup>a</sup>	2.10
	8.64 $\mu$ g/ml SWCNT	86.47 <sup>b</sup>	5.30
	17.28 $\mu$ g/ml SWCNT	97.74 <sup>a, b</sup>	3.10
	34.56 $\mu$ g/ml SWCNT	100.89 <sup>a, b</sup>	2.50
	69.12 $\mu$ g/ml SWCNT	102.06 <sup>a, b</sup>	4.40
	138.24 $\mu$ g/ml SWCNT	99.48 <sup>a, b</sup>	1.80
% pupation*	0 $\mu$ g/ml SWCNT	99.36 <sup>a</sup>	0.40
	8.64 $\mu$ g/ml SWCNT	100.00 <sup>a, b</sup>	0.00
	17.28 $\mu$ g/ml SWCNT	95.87 <sup>a, b</sup>	1.30
	34.56 $\mu$ g/ml SWCNT	96.67 <sup>a, b</sup>	1.20
	69.12 $\mu$ g/ml SWCNT	94.00 <sup>b</sup>	2.50
	138.24 $\mu$ g/ml SWCNT	96.00 <sup>a, b</sup>	1.40
% survival	0 $\mu$ g/ml SWCNT	97.65	0.94
	8.64 $\mu$ g/ml SWCNT	100.0	0
	17.28 $\mu$ g/ml SWCNT	96.76	1.67
	34.56 $\mu$ g/ml SWCNT	95.45	1.73
	69.12 $\mu$ g/ml SWCNT	91.90	2.76
	138.24 $\mu$ g/ml SWCNT	95.17	1.64
Egg/Adult*	0 $\mu$ g/ml SWCNT	19.7	3.00
	17.28 $\mu$ g/ml SWCNT	5.7	2.40
	34.56 $\mu$ g/ml SWCNT	18.9	3.00
	69.12 $\mu$ g/ml SWCNT	16.4	4.10
	138.24 $\mu$ g/ml SWCNT	10.1	2.20

8: Mean ( $\pm$ SE) conversion ratio (percentage of  $\mu\text{g}$  growth for  $\mu\text{g}$  food consumption for each larva) of DBM feeding on artificial food without or with SWCNTs (8.64  $\mu\text{g}/\text{ml}$  and 17.28  $\mu\text{g}/\text{ml}$ ) from feeding trial 1. For this trial, conversion ( $\text{df} = 2$ ;  $F = 7.53$  and  $p = 0.012$ ) was significantly affected by the presence of SWCNTs in the food. Different letters (a- c) represent significant differences in means within a measured variable and between control and different SWCNT concentrations as determined using Tukey's test.

Variable	Treatment	Mean	SE Mean
Conversion*	0 $\mu\text{g}/\text{ml}$ SWCNT	6.771 <sup>a</sup>	0.142
	8.64 $\mu\text{g}/\text{ml}$ SWCNT	5.465 <sup>b</sup>	0.40
	17.28 $\mu\text{g}/\text{ml}$ SWCNT	5.466 <sup>b</sup>	0.22

9: Mean ( $\pm$ SE) conversion ratio (percentage of  $\mu\text{g}$  growth for  $\mu\text{g}$  food consumption for each larva) of DBMs feeding on artificial food without or with SWCNTs (8.64  $\mu\text{g}/\text{ml}$  and 17.28  $\mu\text{g}/\text{ml}$ ) from feeding trial 2. For this trial, conversion ( $\text{df} = 2$ ;  $F = 0.00$  and  $p = 0.998$ ) wasn't significantly affected by the presence of SWCNTs in the food. Different letters (a- c) represent significant differences in means within a measured variable and between control and different SWCNT concentrations as determined using Tukey's test.

Variable	Treatment	Mean	SE Mean
Conversion	0 $\mu\text{g}/\text{ml}$ SWCNT	5.994	0.42
	8.64 $\mu\text{g}/\text{ml}$ SWCNT	6.007	0.36
	17.28 $\mu\text{g}/\text{ml}$ SWCNT	5.970	0.41



10: Mean ( $\pm$ SE) conversion ratio (percentage of  $\mu\text{g}$  growth for  $\mu\text{g}$  food consumption for each larva) of DBMs feeding on artificial food without or with SWCNTs (17.28  $\mu\text{g}/\text{ml}$  and 34.56  $\mu\text{g}/\text{ml}$ ) from feeding trial 3. For this trial, conversion ( $\text{df} = 2$ ;  $F = 0.18$  and  $p = 0.835$ ) wasn't significantly affected by the presence of SWCNTs in the food. Different letters (a- c) represent significant differences in means within a measured variable and between control and different SWCNT concentrations as determined using Tukey's test.

Variable	Treatment	Mean	SE Mean
Conversion	0 $\mu\text{g}/\text{ml}$ SWCNT	5.544	0.27
	17.28 $\mu\text{g}/\text{ml}$ SWCNT	5.471	0.27
	34.56 $\mu\text{g}/\text{ml}$ SWCNT	5.326	0.24

11: Mean ( $\pm$ SE) conversion ratio (percentage of  $\mu\text{g}$  growth for  $\mu\text{g}$  food consumption for each larva) of DBMs feeding on artificial food without or with SWCNTs (17.28  $\mu\text{g}/\text{ml}$ , 34.56  $\mu\text{g}/\text{ml}$  and 69.12  $\mu\text{g}/\text{ml}$ ) from feeding trial 4. For this trial, conversion ( $\text{df} = 3$ ;  $F = 2.2$  and  $p = 0.128$ ) wasn't significantly affected by the presence of SWCNTs in the food. Different letters (a- c) represent significant differences in means within a measured variable and between control and different SWCNT concentrations as determined using Tukey's test.

Variable	Treatment	Mean	SE Mean
Conversion	0 $\mu\text{g}/\text{ml}$ SWCNT	7.642	0.27
	17.28 $\mu\text{g}/\text{ml}$ SWCNT	8.297	0.12
	34.56 $\mu\text{g}/\text{ml}$ SWCNT	8.022	0.22
	69.12 $\mu\text{g}/\text{ml}$ SWCNT	7.846	0.17

12: Mean ( $\pm$ SE) conversion ratio (percentage of  $\mu\text{g}$  growth for  $\mu\text{g}$  food consumption for each larva) of DBMs feeding on artificial food without or with SWCNTs (34.56  $\mu\text{g/ml}$ , 69.12  $\mu\text{g/ml}$  and 138.24  $\mu\text{g/ml}$ ) from feeding trial 5. For this trial, conversion ( $\text{df} = 3$ ;  $F = 11.29$  and  $p \leq 0.0001$ ) was significantly affected by the presence of SWCNTs in the food. Different letters (a- c) represent significant differences in means within a measured variable and between control and different SWCNT concentrations as determined using Tukey's test.

Variable	Treatment	Mean	SE Mean
Conversion***	0 $\mu\text{g/ml}$ SWCNT	8.948 <sup>a</sup>	0.27
	34.56 $\mu\text{g/ml}$ SWCNT	7.376 <sup>b, c</sup>	0.45
	69.12 $\mu\text{g/ml}$ SWCNT	6.117 <sup>c</sup>	0.38
	138.24 $\mu\text{g/ml}$ SWCNT	7.651 <sup>a, b</sup>	0.29

13: Mean ( $\pm$ SE) conversion ratio (percentage of  $\mu\text{g}$  growth for  $\mu\text{g}$  food consumption for each larva) of DBMs feeding on artificial food without or with SWCNTs (138.24  $\mu\text{g/ml}$ ) from feeding trial 6. For this trial, conversion ( $\text{df} = 1$ ;  $F = 1.02$  and  $p = 0.342$ ) wasn't significantly affected by the presence of SWCNTs in the food. Different letters (a- c) represent significant differences in means within a measured variable and between control and different SWCNT concentrations as determined using Tukey's test.

Variable	Treatment	Mean	SE Mean
Conversion	0 $\mu\text{g/ml}$ SWCNT	7.642	0.42
	138.24 $\mu\text{g/ml}$ SWCNT	6.920	0.58

14: Mean ( $\pm$ SE) conversion ratio (percentage of  $\mu\text{g}$  growth for  $\mu\text{g}$  food consumption for each larva) of DBMS feeding on artificial food without or with SWCNTs (8.64  $\mu\text{g}/\text{ml}$ , 17.28  $\mu\text{g}/\text{ml}$ , 34.56  $\mu\text{g}/\text{ml}$ , 69.12  $\mu\text{g}/\text{ml}$  and 138.24  $\mu\text{g}/\text{ml}$ ) from all combined feeding trial. Conversion ( $\text{df} = 5$ ;  $F = 2.33$  and  $p = 0.049$ ) was significantly affected by the presence of SWCNTs in the food. Different letters (a- c) represent significant differences in means within a measured variable and between control and different SWCNT concentrations as determined using Tukey's test.

Variable	Treatment	Mean	SE Mean
Conversion*	0 $\mu\text{g}/\text{ml}$ SWCNT	6.986	0.25
	8.64 $\mu\text{g}/\text{ml}$ SWCNT	5.736	0.27
	17.28 $\mu\text{g}/\text{ml}$ SWCNT	6.238	0.29
	34.56 $\mu\text{g}/\text{ml}$ SWCNT	6.492	0.34
	69.12 $\mu\text{g}/\text{ml}$ SWCNT	6.982	0.34
	138.24 $\mu\text{g}/\text{ml}$ SWCNT	7.285	0.33

15: Mean ( $\pm$ SE) consumption, growth, pupation, and survival of DBMs feeding on artificial food without or with AgNP (4.32  $\mu$ g/ml and 8.64  $\mu$ g/ml) from feeding trial 1. For this trial, growth (df = 2; F = 2.70 and p = 0.107), survival (df = 2; F = 1.35 and p = 0.297) and egg/adult (df = 2; F = 1.34 and p = 0.299) wasn't significantly affected by the presence of AgNPs in the food. Consumption (df = 2; F = 6.28 and p = 0.014) and pupation (df = 2; F = 10.00 and p = 0.003) was significantly affected by the presence of AgNPs in the food. Different letters (a- c) represent significant differences in means within a measured variable and between control and different AgNP concentrations as determined using Tukey's test.

Variable	Treatment	Mean	SE Mean
Consumption/L/H ( $\mu$ g) *	0 $\mu$ g/ml AgNP	1462.22 <sup>a</sup>	69.70
	4.32 $\mu$ g/ml AgNP	1309.96 <sup>a, b</sup>	61.30
	8.64 $\mu$ g/ml AgNP	1182.54 <sup>b</sup>	27.30
Growth/L/H ( $\mu$ g)	0 $\mu$ g/ml AgNP	110.67	2.95
	4.32 $\mu$ g/ml AgNP	92.90	9.61
	8.64 $\mu$ g/ml AgNP	87.12	8.13
% pupation **	0 $\mu$ g/ml AgNP	100.00 <sup>a</sup>	0.00
	4.32 $\mu$ g/ml AgNP	100.00 <sup>a</sup>	0.00
	8.64 $\mu$ g/ml AgNP	90.00 <sup>b</sup>	3.16
% Survival	0 $\mu$ g/ml AgNP	98.00	1.33
	4.32 $\mu$ g/ml AgNP	97.00	2.13
	8.64 $\mu$ g/ml AgNP	97.64	1.58
Egg/Adult	0 $\mu$ g/ml AgNP	19.74	5.69
	4.32 $\mu$ g/ml AgNP	40.20	10.10
	8.64 $\mu$ g/ml AgNP	33.43	10.40

16: Mean ( $\pm$ SE) consumption, growth, pupation, and survival of DBMs feeding on artificial food without or with AgNP (4.32  $\mu$ g/ml and 8.64  $\mu$ g/ml) from feeding trial 2. For this trial, growth (df = 2; F = 1.25 and p = 0.321), pupation (df = 2; F = 0.50 and p = 0.619), survival (df = 2; F = 2.25 and p = 0.148) and egg/adult (df = 2; F = 0.00 and p = 0.997) was not significantly affected by the presence of AgNPs in the food. Consumption (df = 2; F = 43.16 and p  $\leq$  0.0001) was significantly affected by the presence of AgNPs in the food. Different letters (a- c) represent significant differences in means within a measured variable and between control and different AgNP concentrations as determined using Tukey's test.

Variable	Treatment	Mean	SE Mean
Consumption/L/H ( $\mu$ g) *	0 $\mu$ g/ml AgNP	1617.5 <sup>a</sup>	24.9
	4.32 $\mu$ g/ml AgNP	1317.6 <sup>c</sup>	20.6
	8.64 $\mu$ g/ml AgNP	1456.0 <sup>b</sup>	22.8
Growth/L/H ( $\mu$ g)	0 $\mu$ g/ml AgNP	81.41	5.83
	4.32 $\mu$ g/ml AgNP	68.62	5.60
	8.64 $\mu$ g/ml AgNP	75.44	5.75
% pupation	0 $\mu$ g/ml AgNP	100.00	0.00
	4.32 $\mu$ g/ml AgNP	98.00	2.00
	8.64 $\mu$ g/ml AgNP	98.00	2.00
% survival	0 $\mu$ g/ml AgNP	96.0	2.45
	4.32 $\mu$ g/ml AgNP	100.0	0.0
	8.64 $\mu$ g/ml AgNP	95.28	2.90
Egg/Adult	0 $\mu$ g/ml AgNP	29.52	7.65
	4.32 $\mu$ g/ml AgNP	28.91	3.70
	8.64 $\mu$ g/ml AgNP	29.32	5.00

17: Mean ( $\pm$ SE) consumption, growth, pupation, and survival of DBMs feeding on artificial food without or with AgNP (4.32  $\mu$ g/ml and 8.64  $\mu$ g/ml) from all combined feeding trial. Growth (df = 2; F = 2.19 and p = 0.131), % survival (df = 2; F = 0.09 and p = 0.917) and egg/adult (df = 2; F = 0.93 and p = 0.405) was not significantly affected by the presence of AgNPs in the food. Consumption (df = 2; F = 9.64 and p = 0.001) and pupation (df = 2; F = 5.26 and p = 0.012) was significantly affected by the presence of AgNPs in the food. Different letters (a- c) represent significant differences in means within a measured variable and between control and different AgNP concentrations as determined using Tukey's test.

Variable	Treatment	Mean	SE Mean
Consumption/L/H ( $\mu$ g) **	0 $\mu$ g/ml AgNP	1539.9 <sup>a</sup>	43.50
	4.32 $\mu$ g/ml AgNP	1313.8 <sup>b</sup>	30.50
	8.64 $\mu$ g/ml AgNP	1319.3 <sup>b</sup>	48.60
Growth/L/H ( $\mu$ g)	0 $\mu$ g/ml AgNP	96.04	5.77
	4.32 $\mu$ g/ml AgNP	80.76	6.62
	8.64 $\mu$ g/ml AgNP	81.28	5.08
% pupation*	0 $\mu$ g/ml AgNP	100.00 <sup>a</sup>	0.00
	4.32 $\mu$ g/ml AgNP	99.00 <sup>a</sup>	1.00
	8.64 $\mu$ g/ml AgNP	94.00 <sup>b</sup>	2.21
% survival	0 $\mu$ g/ml AgNP	100.0	0.0
	4.32 $\mu$ g/ml AgNP	94.0	4.0
	8.64 $\mu$ g/ml AgNP	100.0	0.0
Egg/Adult	0 $\mu$ g/ml AgNP	24.63	4.78
	4.32 $\mu$ g/ml AgNP	34.56	5.43
	8.64 $\mu$ g/ml AgNP	31.37	5.50

18: Mean ( $\pm$ SE) conversion ratio (percentage of  $\mu\text{g}$  growth for  $\mu\text{g}$  food consumption for each larva) of DBMs feeding on artificial food without or with AgNP (4.32  $\mu\text{g}/\text{ml}$  and 8.64  $\mu\text{g}/\text{ml}$ ) from feeding trial 1. For this trial, conversion ( $\text{df} = 2$ ;  $F = 0.23$  and  $p = 0.798$ ) wasn't significantly affected by the presence of AgNPs in the food. Different letters (a- c) represent significant differences in means within a measured variable and between control and different AgNP concentrations as determined using Tukey's test.

Variable	Treatment	Mean	SE Mean
Conversion	0 $\mu\text{g}/\text{ml}$ AgNP	7.64	0.42
	4.32 $\mu\text{g}/\text{ml}$ AgNP	7.10	0.71
	8.64 $\mu\text{g}/\text{ml}$ AgNP	7.32	0.54

19: Mean ( $\pm$ SE) conversion ratio (percentage of  $\mu\text{g}$  growth for  $\mu\text{g}$  food consumption for each larva) of DBMs feeding on artificial food without or with AgNPs (4.32  $\mu\text{g}/\text{ml}$  and 8.64  $\mu\text{g}/\text{ml}$ ) from feeding trial 2. For this trial, conversion ( $\text{df} = 2$ ;  $F = 0.08$  and  $p = 0.927$ ) wasn't significantly affected by the presence of AgNPs in the food. Different letters (a- c) represent significant differences in means within a measured variable and between control and different AgNP concentrations as determined using Tukey's test.

Variable	Treatment	Mean	SE Mean
Conversion	0 $\mu\text{g}/\text{ml}$ AgNP	5.02	0.31
	4.32 $\mu\text{g}/\text{ml}$ AgNP	5.23	0.48
	8.64 $\mu\text{g}/\text{ml}$ AgNP	5.18	0.38

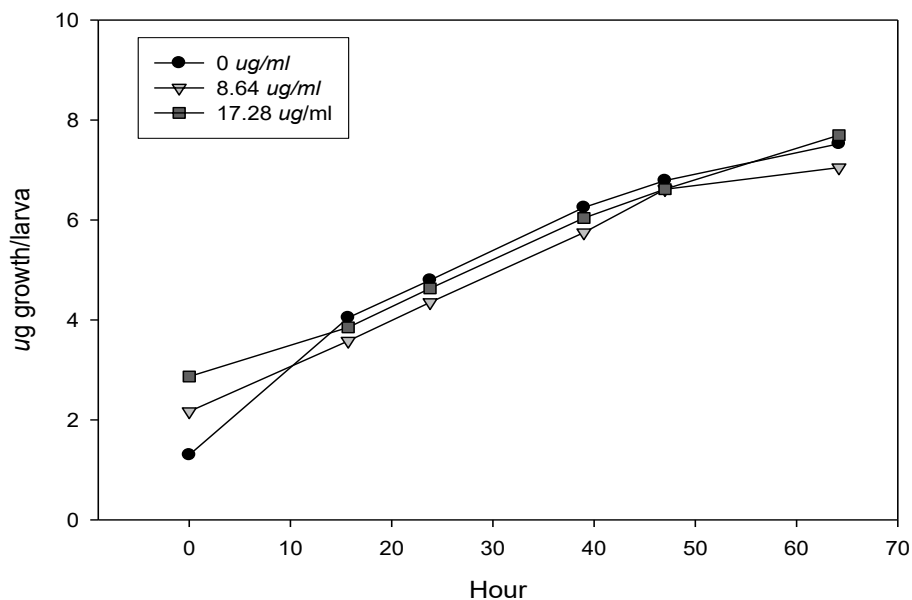
20: Mean ( $\pm$ SE) conversion ratio (percentage of  $\mu$ g growth for  $\mu$ g food consumption for each larva) of DBMs feeding on artificial food without or with AgNP (4.32  $\mu$ g/ml and 8.64  $\mu$ g/ml) from all combined feeding trial. Conversion ( $df = 2$ ;  $F = 0.03$  and  $p = 0.972$ ) wasn't significantly affected by the presence of AgNPs in the food. Different letters (a- c) represent significant differences in means within a measured variable and between control and different AgNP concentrations as determined using Tukey's test.

Variable	Treatment	Mean	SE Mean
Conversion	0 $\mu$ g/ml AgNP	6.33	0.50
	4.32 $\mu$ g/ml AgNP	6.16	0.51
	8.64 $\mu$ g/ml AgNP	6.25	0.47

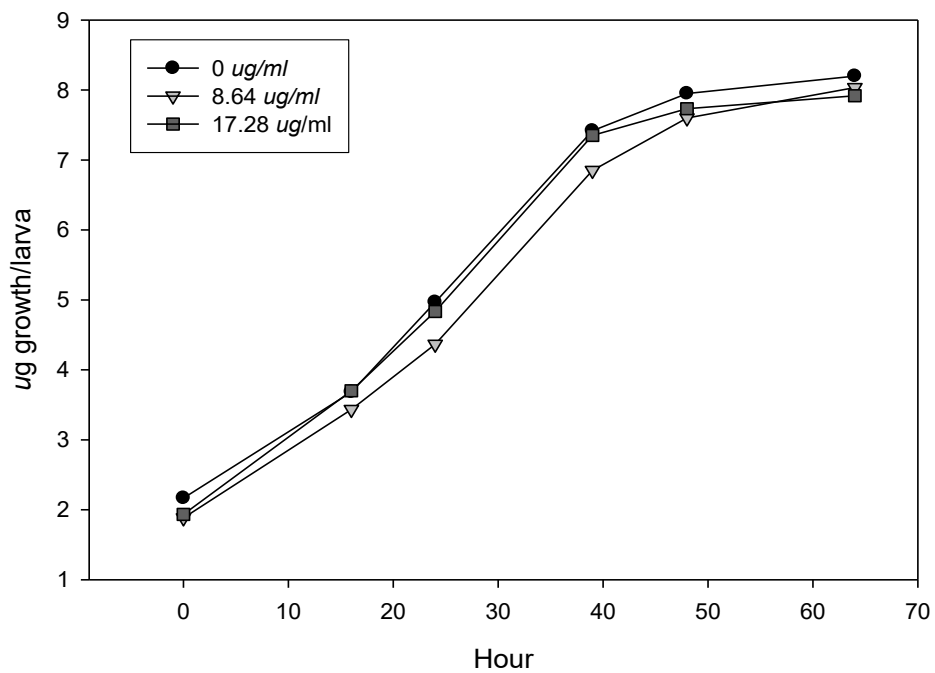
21: Mean ( $\pm$ SE) gene expression of CYP6BG1 of DBMs feeding on artificial food without or with SWCNT (138.24  $\mu$ g/ml) and AgNP (4.32  $\mu$ g/ml and 8.64  $\mu$ g/ml) from feeding trial 6 and 1. Gene expression ( $df = 3$ ;  $F = 1.43$  and  $p = 0.252$ ) wasn't significantly affected by the presence of SWCNT and AgNP in the food.

Variable	Treatment	Mean	SE Mean
Fold change of gene	0 $\mu$ g/ml	1.0	0.0
	138.24 $\mu$ g/ml SWCNT	1.43	0.47
	4.32 $\mu$ g/ml AgNP	2.53	0.76
	8.64 $\mu$ g/ml AgNP	1.44	0.62

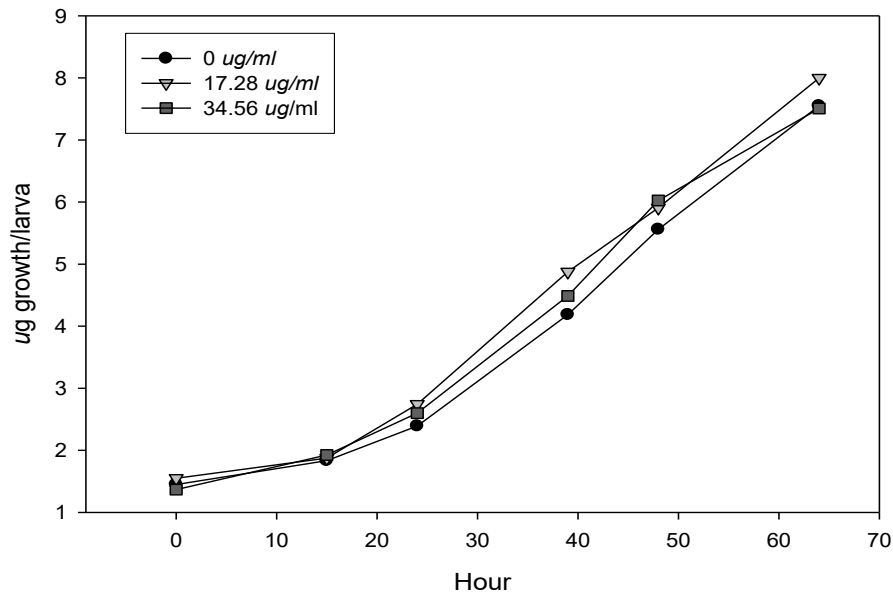




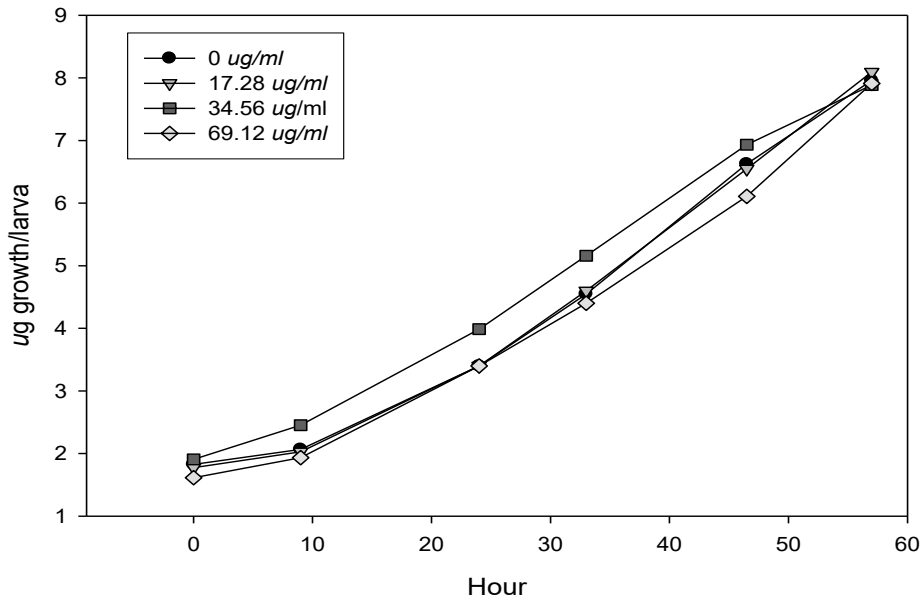
22: Larval growth ( $\mu\text{g}$ ) along with time for trial 1 for control and different SWCNT concentration food.



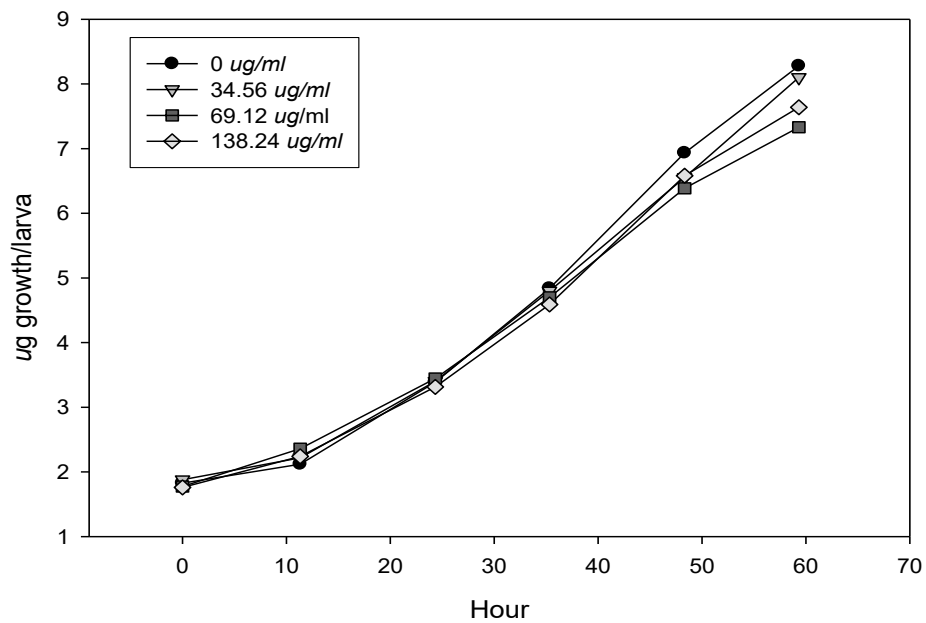
23: Larval growth ( $\mu\text{g}$ ) along with time for trial 2 for control and different SWCNT concentration food



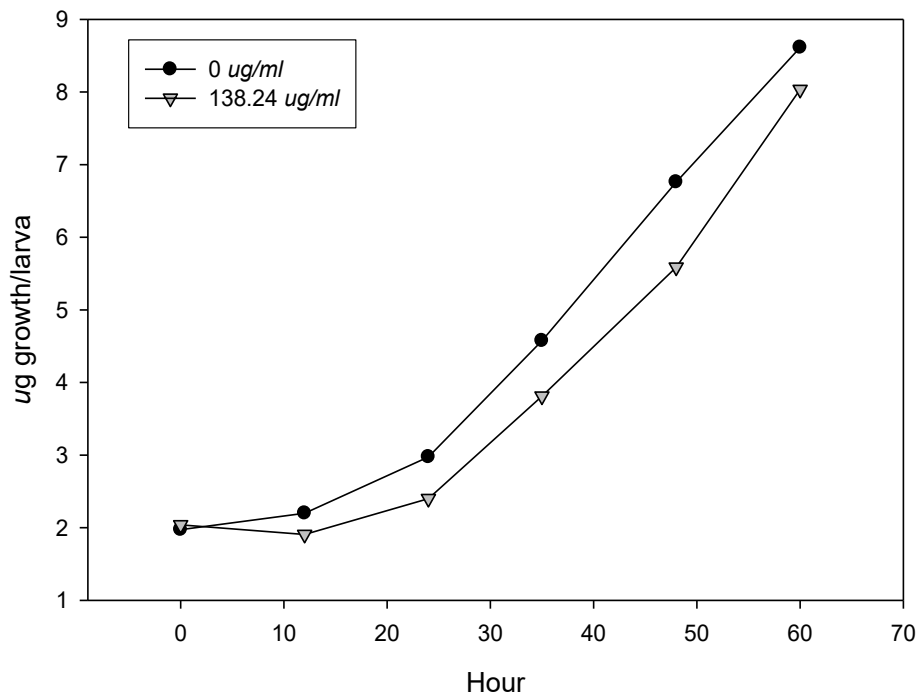
24: Larval growth ( $\mu\text{g}$ ) along with time for trial 3 for control and different SWCNT concentration food



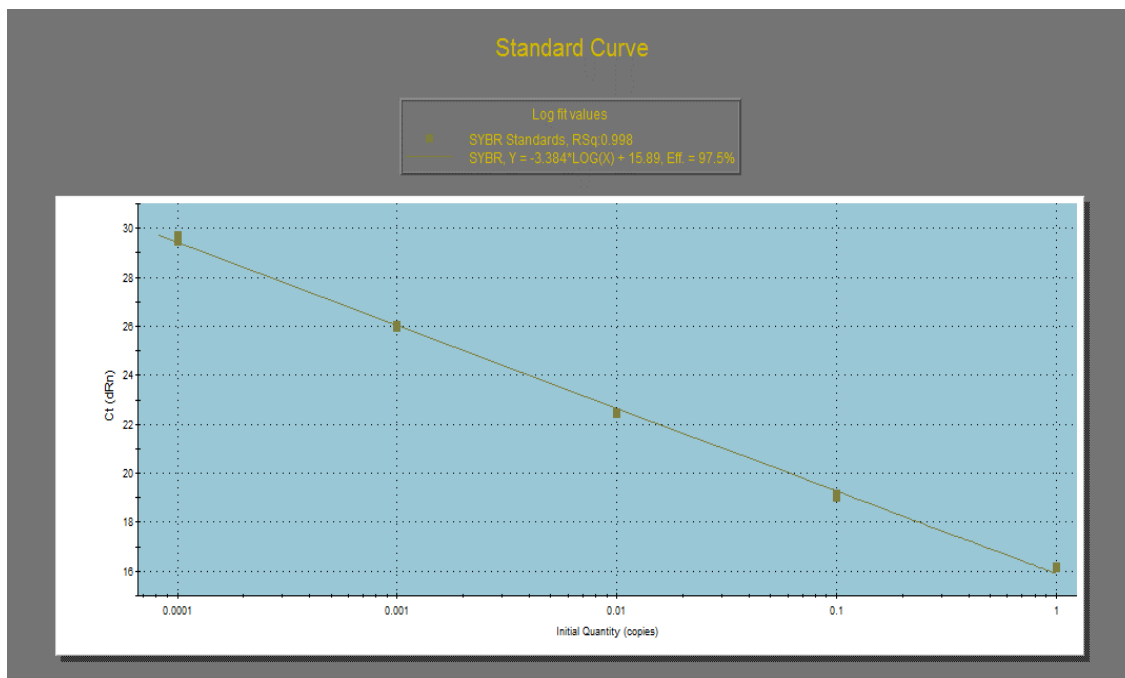
25: Larval growth ( $\mu\text{g}$ ) along with time for trial 4 for control and different SWCNT concentration food



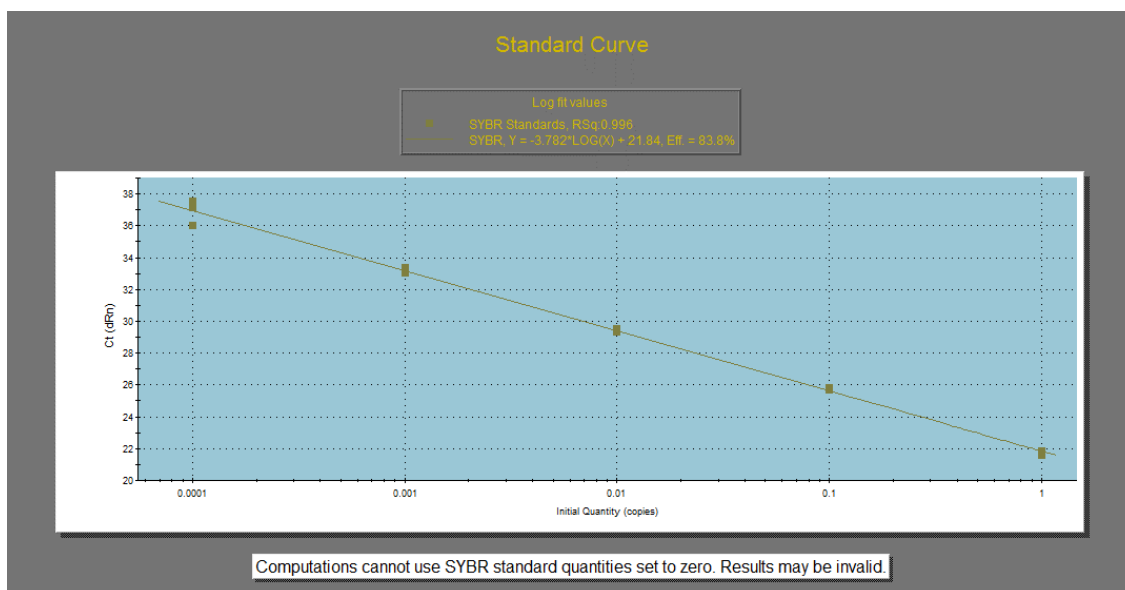
26: Larval growth ( $\mu\text{g}$ ) along with time for trial 5 for control and different SWCNT concentration food



27: Larval growth ( $\mu\text{g}$ ) along with time for trial 6 for control and different SWCNT concentration food



28: Standard curve for reference gene RPL 32



29: Standard curve for target gene CYP6BG1